

ADVANCES IN
Agronomy

VOLUME 89



ADVANCES IN *A*gronomy

VOLUME 89



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VOLUME 89



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Preface

Volume 89 contains six comprehensive and timely reviews. Chapter 1 presents a thorough coverage of wet chemistry and state-of-the-art molecular scale techniques, such as x-ray absorption fine structure (XAFS) and nuclear magnetic resonance (NMR) spectroscopies, that can be used to characterize phosphorus in organic wastes. Chapter 2 discusses the Wheat Genetics Resource Center that has served the scientific community for 25 years. These resources have been useful to scientists in 45 countries and 39 of the states in the U.S. Chapter 3 covers various aspects of the biology and management of *Stevia*, a sweet herb of Paraguay. Chapter 4 is a timely review of aspects of soil fertility decline in the tropics as assessed by soil chemical measurements. Chapter 5 covers nematode interactions and assessment of models for their control on crop plants. Chapter 6 presents data and algorithms on ammonia emission from animal operations, a current area of much interest in the area of environmental quality.

I am grateful for the authors' excellent reviews.

DONALD L. SPARKS
EDITOR

ADVANCES IN THE CHARACTERIZATION OF PHOSPHORUS IN ORGANIC WASTES: ENVIRONMENTAL AND AGRONOMIC APPLICATIONS

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- II. Types of Organic Wastes
 - A. Agricultural Wastes
 - B. Municipal Wastes
- III. Factors Affecting Phosphorus Composition in Organic Wastes
 - A. Dietary Effects
 - B. Organic Wastes Handling Effects
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 - A. Total Phosphorus
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 - F. Nuclear Magnetic Resonance Spectroscopy
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- V. Summary
- References

There is international interest today in the fate and transformation of phosphorus (P) applied to soils due to historical overapplication of P from organic wastes. This overapplication has increased soil solution P concentrations and enriched the erodible fraction of soil with P. This is of major concern as significant water quality deterioration can occur if P applied to

soils in organic wastes reaches water bodies. Just as the bioavailability of P compounds depends upon their chemical form, it is becoming increasingly apparent that information about different forms of P is needed for holistic management of organic wastes. A number of chemical and biological methods have been employed to partition total P into more specific chemical forms in organic wastes. However, there has been no previous effort to review and synthesize the literature and to critically analyze the various techniques with promise for chemical speciation of P in organic wastes. In this chapter, we review various types of organic wastes and factors affecting P composition in organic wastes, from production to land disposal. Then, we discuss the various methods that have been used to characterize P forms, including water extractable P (WEP) physicochemical fractionation, sequential chemical fractionation, enzymatic hydrolysis, nuclear magnetic resonance (NMR), and x-ray absorption near edge structure (XANES) spectroscopy. To summarize the conclusions, WEP is quick chemical test that should be employed to determine the readily dissolved P in organic wastes and to assess the potential risk of wastes on water quality. The potential bioavailability of P forms in the liquid wastes can be similarly assessed by a rapid and low cost physicochemical fractionation method. Enzymatic hydrolysis and solution state NMR can be of great benefit to characterize organic P species in wastes, whereas solid-state NMR and XANES spectroscopy are better suited to study the inorganic P minerals in the wastes. NMR and XANES methods are both quantitative and can be used to study the influence of management practices on P speciation. Solid-state NMR and XANES methods are capable of performing analysis of heterogeneous material and provides complementary information about P compounds in organic wastes. The combined use of sequential chemical fractionation and spectroscopic methods (NMR, XANES) allows for accurate identification of P compounds in the sequential extracts. Case studies are included throughout the chapter to discuss wider applicability of a particular method. We conclude this chapter by suggesting that more than one method may be necessary for complete determination of P species in organic wastes.

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I. INTRODUCTION

Eutrophication of water bodies can result in the death of fish and other marine animals, cessation of recreational activities, appearance of harmful algal blooms, and degradation of the safety and quality of drinking water supplies (Burkholder *et al.*, 1992; Glasgow *et al.*, 2001; Kotak *et al.*, 1993). The contribution of agriculture to environmental problems associated with phosphorus (P) is significant in many regions of the world (Table I). For example, in the United Kingdom, 89% of nitrogen (N) and P enrichment in the water bodies has been reported to be from agricultural sources. Instances of nonpoint P pollution are widespread and include the Chesapeake Bay,

Table I
Contribution of Agriculture to Environmental Problems in Selected European Countries^a

Name of the country	Environmental impact	Contribution (%)
UK	N and P in water	89
The Netherlands	Eutrophication	80
Belgium	Enrichment of soil and water with N and P	66
Germany	P inputs to surface water	48
Denmark	Emission of P to the sea	26

^aAdapted from De Clerq *et al.* (2001).

Lake Washington and the Great Lakes in the United States, Gippsland Lakes in Victoria, Australia, and the Alpine Lakes of Italy and Switzerland. These instances of eutrophication have forced environmental authorities in some countries to devise new rules and regulations to combat the accelerated eutrophication of surface water bodies caused by P losses from agricultural lands. Several countries have established water quality criteria for P in freshwater ecosystems. For example, in the United Kingdom, Moss *et al.* (1988) stated that most eutrophication problems in lake systems occur when total dissolved P concentrations exceed $30 \mu\text{g liter}^{-1}$, while a critical concentration limit of $100 \mu\text{g total P liter}^{-1}$ has been proposed for river systems (English Nature, 1994). The US Environmental Protection Agency (2002) established ecoregional nutrient criteria that divide the whole country into 14 ecoregions based on properties of the water bodies. According to this approach, maximum total P concentrations of $8\text{--}38 \mu\text{g liter}^{-1}$ are permissible for lakes and reservoirs, while for rivers and streams, the permissible total P concentrations are $10\text{--}128 \mu\text{g liter}^{-1}$.

The continuous overapplication of organic wastes to soils has resulted in increasing concentrations of P in the soil solution and enriching the erodible fraction with P. It is also known that background losses of P from these overfertilized soils are often above the concentrations of P required for eutrophication. Therefore, the transfer of environmentally significant quantities of P from land to aquatic systems is difficult to avoid in landscapes. Also, there is international interest today in the fate and transformations of P applied to soils in organic wastes, such as animal manures, municipal biosolids, and industrial by-products. The growing interest in this topic stems from: (i) long-standing agricultural concerns about the most efficient means to beneficially recycle the P in organic wastes as a plant nutrient and (ii) increased regulation of all forms of organic P sources used as soil amendments in order to prevent nonpoint source pollution of surface and shallow ground waters.

The typical approach used by land managers to characterize P in organic wastes is to measure "total P." This is usually accomplished by some form of acid digestion, followed by colorimetric or inductively coupled plasma-optical

emission spectroscopy (ICP-OES) analysis (Murphy and Riley, 1962; US Environmental Protection Agency, 1986). Most recently, there has been growing interest in measuring “water extractable P” (WEP), to determine the potential effect of land application of organic wastes on dissolved P losses via surface runoff and leaching. Efforts are now underway to develop standard tests to measure WEP in organic wastes (Kleinman *et al.*, 2002; Wolf *et al.*, 2005) and biosolids (Brandt *et al.*, 2004) and to interpret the results of these tests, however, progress has been slow and somewhat fragmented. At the same time, scientists conducting research on fate and transformations of P applied in organic wastes are well aware that the plant availability and environmental fate of P is strongly influenced by the nature and relative distribution of the P species present in these materials. Most scientists involved in this research recognize that measuring total P or WEP will provide only limited information about the fate of P in soils amended with organic wastes.

Fortunately, research has begun to advance our knowledge of the speciation of P in organic wastes by applying new analytical methodologies, such as solution and solid state nuclear magnetic resonance (NMR) (Hunger *et al.*, 2004, 2005; Maguire *et al.*, 2004; Toor *et al.*, 2005a; Turner and Leytem, 2004), and x-ray absorption near edge structure (XANES) spectroscopy (Peak *et al.*, 2002; Toor *et al.*, 2005c). At the same time there is a growing body of literature from both agronomic and environmental perspectives on the use of chemical sequential fractionation (Barnett, 1994a; Dou *et al.*, 2000; Sharpley and Moyer, 2000) and biochemical enzyme hydrolysis techniques (He *et al.*, 2003b, 2004b) to characterize the forms of P in various organic wastes. These studies have provided detailed information about inorganic and organic P forms in wastes and pointed out that present day manures have higher concentrations of inorganic P (up to 70% of total P) compared with past manures (40–50% of total P) (Barnett, 1994b; Funatsu, 1908; Tsuda, 1909). This is mainly attributed to changes in the nature of animal diets, which now include more concentrates and mineral supplements. In addition, enzyme additives, such as phytase, are increasingly being added to poultry and swine diets to increase dietary P utilization, which also results in the conversion of dietary organic P into manure inorganic P. We believe that there is a need to review and synthesize the literature in this area and to critically analyze the various approaches now being used, or potentially available, to characterize P in organic wastes.

Therefore, the objectives of this chapter are:

1. Review the scientific literature on the characterization of P in organic wastes focusing on manures and biosolids (sewage sludge). What has been done to date in this regard and how are “traditional” methods useful in our efforts to develop environmentally sound land management strategies for organic wastes?

2. What advances have occurred in recent research (e.g., the past 10 years) on the characterization of P in organic wastes that can provide us with a more complete understanding of the transformations and potential plant availability and mobility of P when these materials are used as soil amendments?
3. What should land managers do today to best characterize organic wastes, for their potential agronomic value and their possible effects on water quality?

II. TYPES OF ORGANIC WASTES

Organic wastes can be broadly grouped into following two categories.

A. AGRICULTURAL WASTES

Over the last four decades, a considerable increase in the number of domesticated animals has occurred throughout the world (Table II). For example, cattle and buffalo population increased by 42 and 95%, and increase of 124, 134, and 315% were recorded for the goats, pigs, and chickens, respectively. This increase has been accompanied by a parallel increase in the wastes produced by these animals. The annual average generation of animal solid manure in the United States from beef cattle is 24.4 million Mg, followed by 19 million Mg of dairy, 14.5 million Mg of swine, and 12.7 million Mg of poultry litter and manure (Walker *et al.*, 1997). These animal wastes contain 2.3 million Mg of P, which is 0.7 million Mg higher than the amount of P applied to soils in commercial fertilizers (1.6 Mg P) (Wright *et al.*, 1998).

Table II
World Livestock Population^a

	1961	2004	Increase (%)
Sheep (<i>Ovis aries</i>)	994,268,736	1,038,765,370	4
Cattle (<i>Bos primigenius</i>)	941,715,069	1,334,501,290	42
Buffaloes (<i>Bos bubalus</i>)	88,505,407	172,719,487	95
Turkeys (<i>Meleagris gallopavo</i>)	130,745	276,225	111
Goats (<i>Capra hircus</i>)	348,726,793	780,099,948	124
Pigs (<i>Sus domesticus</i>)	406,190,364	951,771,892	134
Chickens (<i>Gallus domesticus</i>)	3,898,045	16,194,925	315
Ducks (<i>Anas platyrhynchos</i>)	186,756	1,019,479	446

^aAdapted from FAO (2004).

Animal manures can be grouped, based on their moisture content, into three broad categories: solid, semisolid, slurry or liquid. Manures that have greater than 20% solids can be handled as a solid. For example, poultry litter (mixture of feces and bedding material) will usually have 70% or more solids. Manures with 10–20% solids fall in the semisolid category and are represented by most dairy farm wastes. Manures with less than 10% but greater than 4% solids can be treated as slurry. These are typical of deep swine lagoon pits; however, dairy manure with milking parlor washwater is also handled as slurry. Liquid manure with less than 4% solids can be handled with common irrigation equipment. Properly designed and managed lagoon pits and wastes generated from washing of dairy milking operations (milkhouse and milk parlor) will have typically less than 1% solids. Table III includes most common types of manures (solid, lagoon,

Table III
Estimated Solid, Semisolid (Lagoon), and Liquid Manure and Total Phosphorus Produced per Animal per Year (in kg year⁻¹) in the United States^a

Animal	Livestock stage	Solid		Semisolid (Lagoon)		Liquid	
		Manure	Total P	Manure	Total P	Manure	Total P
Dairy	Dairy herd	18,090	15.7	62,100	5.9	32,850	25.8
	Dairy cow	12,600	8.3	40,950	3.7	24,300	19.1
	Dairy heifer	5,850	3.9	—	—	11,250	8.3
	Dairy calf	1,350	1.0	—	—	2,700	2.0
	Veal calf	990	0.6	—	—	1,575	1.8
	Feeder calves	3,150	2.8	—	—	5,850	5.1
	Finishing cattle	5,310	8.1	—	—	11,475	10.8
	Fattening cattle	—	—	19,800	4.1	—	—
Beef	Cow	6,030	5.3	—	—	13,500	11.4
Poultry	Layer	18	0.2	—	—	59	0.2
	Broiler	8	0.1	59	0.01	37	0.1
	Turkey	41	0.2	—	—	231 ^b	0.2 ^c
	Duck	27	0.1	—	—	112.1	0.1
Swine	Farrowing	2,160	2.8	7,425	1.2	5,175	3.3
	Nursery	216	0.4	—	—	450	0.4
	Grow-finish	945	1.8	3,600	0.4	4,275 ^d	2.7 ^e
	Farrow-finish	7,713	13.6	28,800	4.5	16,875	21.2
	Breeding-gestation	900	1.4	5,175	0.8	3,150	4.1
Total		65,357	—	167,909	—	129,358	—

^aAdapted from Mid West Plan Service (1988).

^bSum of Tom and Hen turkeys.

^cAverage of Tom and Hen turkeys.

^dSum of deep pit, wet/dry feeder, and earthen pit.

^eAverage of deep pit, wet/dry feeder, and earthen pit.

and liquid) and their annual generation by dairy, beef, poultry, and swine in the United States. Most of the wastes generated by all animal species (total of dairy, beef, poultry, swine) fall in the semisolid ($167,909 \text{ kg year}^{-1}$) and liquid ($129,358 \text{ kg year}^{-1}$) categories, although dairy, beef, and swine (far-row-finish, farrowing) contribute considerable quantities of solid manure ($65,357 \text{ kg year}^{-1}$).

The discussion on other wastes generated by agricultural operations, including crop residues, food processing wastes, wood harvesting and milling (paper mill sludge, woodchips, sawdust), and public and private horticulture (composts, lawn and leaf clippings) is not covered in this review.

1. Dairy and Beef

Dairy operations produce both solid and liquid wastes. Solid manure is generated from confined dairy or beef facilities, whereas liquid manure (mixture of feces, urine, and washwater) is a waste generated from a dairy parlor and in some confined dairy operations. Large dairy operations (>200 milk cows) and some medium sized farms (80–200 cows) tend to use liquid, rather than solid or semisolid, manure handling systems (Dougherty *et al.*, 1998) because of the efficient automated systems that are employed for watering, cleaning, and sanitizing. Use of automated flushing systems has also reduced the need of bedding materials thereby producing manures with lower solids content. However, smaller livestock farms commonly employ traditional solid or semisolid manure-handling procedures due to the higher costs associated with installing automatic flushing systems. In rotational grazing (mostly for dairy and sheep), manure is naturally spread on land as the animals graze, while in an open feedlot (mostly for beef) manure may be occasionally scraped or temporarily stored as pile before land spreading. The amount and type of bedding material not only affects manure solids content but also alters the physical, chemical, and biological composition of the manure. The most commonly used bedding materials in dairy operations are sand, sawdust, and straw, although some operations use paper sludge or shredded newspaper.

2. Poultry

Major poultry wastes are poultry manure and poultry litter (mixture of poultry manure and bedding material) and are principally generated by broilers, turkeys, layers, and ducks. There are two types of manure generation systems: (i) liquid or semisolid manure is generated from caged pit

systems (layers) where manure falls into a pit and is then either scraped or flushed (no bedding material is used) and (ii) solid manure is generated from floor/litter systems (broilers, turkeys) on earthen or concrete floors covered with bedding material such as sawdust, wood chips, or other materials. In the United States, complete waste removal for broilers is usually accomplished after 12–24 months with partial cleaning after each flock (approximately 49 days). This poultry waste is usually directly applied to land after its removal, but solid manure can also be stored in roofed or covered structures. Field storage of poultry manure as stockpiles or in lagoons and manure pelletizing are also common. Comprehensive reviews of broiler waste generation and issues related to its management can be found in Cabrera and Sims (2000), Sims and Wolf (1994), and Williams *et al.* (1999).

3. Swine

Swine manure is handled as solid or liquid depending upon the type of housing and manure handling system. In the United States, swine are fed diets that are very similar to poultry and that are formulated with corn or grain sorghum and soybean meal. Greater than 50–60% of swine operations in the United States use total confinement systems where hydraulic water flushing systems are used and the manure is typically handled as slurry in anaerobic lagoons. Approximately 15% of the swine raised in the United States have solid manure handling systems, whereas very few (<5%) of the swine are raised on pasture or in open feedlot. The methods of manure collection, dilution, and storage are the major factors affecting composition of nutrients in swine manure.

B. MUNICIPAL WASTES

The major municipal wastes are municipal solid waste (MSW) and biosolids, with the former accounting for 95% of the total (National Research Council, 2002). While the other municipal wastes are wastewaters produced from sewage treatment, MSW composts, and drinking water residuals (solids from drinking water treatment). The MSW is a mixture of paper and cardboard products (35%), yard wastes (20%), and metals, plastic, glass, wood, and food wastes (each comprises about 6–9%). Approximately 60% of MSW is biodegradable (paper, cardboard, food wastes) and can be potentially recycled by means of composting. The term “biosolids” is a relatively new name for sewage sludge. According to the US Environmental Protection Agency (1995), biosolids are “the primarily organic solid product yielded by municipal wastewater treatment processes that can be beneficially recycled.”

Considerable amounts of biosolids are produced each year and this figure is increasing. For example, in 1998 biosolids production in the United States was 6.3 million dry Mg and is expected to increase by 19% (7.5 million dry Mg) in 2010 (US Environmental Protection Agency, 1999). Increases in biosolids production require greater resources to beneficially reuse them by means of land application. In 2000, 40.1% of the biosolids were land applied followed by incineration (21.9%), landfilling (17.2%), composting (5.4%), lime stabilization (4.1%), surface disposal (3.8%), heat drying and pelletization (1.4%), and lagoon storage (0.90%).

III. FACTORS AFFECTING PHOSPHORUS COMPOSITION IN ORGANIC WASTES

Phosphorus forms in organic wastes can be influenced by a number of factors, ranging from the origin (animals, industries) to practices that occur during the generation, treatment, handling, and storage (e.g., the type of P fed to animals, bedding materials, the addition of lime and metal salts to biosolids).

A. DIETARY EFFECTS

Over the last few years, the P content and/or forms of P in some manures has been significantly changed due to changes in animal nutrition. The driving force for these changes has been to increase the cost efficiency of feed consumption, animal performance, and the need to better manage organic wastes by reducing P excretion in manures.

1. Poultry and Swine Diets

Dietary manipulation by feeding P closer to animal requirement and by using feed additives, such as phytase and vitamin D metabolites, is an emerging area of research because imported feedstuffs are the principal P inputs on many farms in the United States and Europe. For example, imported feedstuffs supplied 5859 kg P ha⁻¹ in the form of concentrates on a Belgian pig farm that raised 5000 pigs per year (Table IV). The P surplus on this farm was 1056 kg P ha⁻¹ and was much higher than other European farms (14–41 kg P ha⁻¹). Similarly, concentrates were the major P input on some European dairy farms that had P surpluses of 8–23 kg P ha⁻¹.

Table IV
Farm Gate Phosphorus Balances for Selected European Pig and Dairy Farms^a (in kg P ha⁻¹)

	Pig					Dairy				
	Belgium ^b	Denmark ^c	Spain ^d	France ^e	Sweden ^f	Belgium ^g	Denmark ^h	Spain ⁱ	France ^j	Sweden ^k
Input										
Mineral fertilizer	0	1	21	9	4	13	5	22	6	1
Manure	0	0	0	0	0	0	0	0	13	0
Fodder	0	95	0	94	49	10	43	0	0	17
Concentrates	5859	0	182	0	0	31	0	11	7	0
Animal products	561	0	9	0.4	5	5	0	0	0.2	0
Atmospheric deposition	0	0	1	0	0	1	0	1	0	0
Net stock exchange ^l		-27	0	0	0	0	7	0	0	0
Total input	6420	69	213	103	58	60	55	34	27	18
Output										
Organic fertilizers	2945	0	141	40	0	12	0	0	0	0
Arable products	0	16	12	0	0	2	23	18	7	1
Animals and animal products	2355	30	46	49	17	23	10	5	7	9
Other	64	0	0	0	0	0.2	0	0	0	0
Total output	5364	46	199	89	17	37	33	23	14	10
Surplus	1056	23	14	14	41	23	22	11	13	8
Utilization (%)	84	67	93	86	29	61	60	68	52	55

^aAdapted from De Clerq *et al.* (2001).

^bFarm with no agricultural land and 2000 fattening pigs, with 2.5 cycles per year.

^cMeans of 13 farms with total area of 79 ha (7 ha of grassland, 1 ha of fodder, 57 ha of cereals, 9 ha of oilseed rape and field pea, 4 ha of fallow). Eight of the pig farms produced piglets at an average of 22 piglets per sow per year and housed an average of 387 sows. The remaining five farms housed 216 sows with a mean annual production of 3988 pigs.

^dFarm with 14 ha of rainfed barley and 1018 pigs in a 5 month cycle.

^eFarm with 27 ha of wheat, 22 ha of maize, and 220 sows. Farm sold 3920 fattening pigs in 1999. Farm exports 1250 m³ of slurry per year to neighboring farms due to animal waste management constraints.

^fFarm with 73 ha of arable cropland and 2300 fattening pigs per year.

^gFarm with 33 ha of grassland, no arable crops, and 39 dairy cows.

^hMeans of 26 farms with total area of 85 ha (27 ha of grassland, 26 ha of fodder, 23 ha of cereals, 7 ha of beet, 2 ha of fallow) and 128 dairy cows.

ⁱFarm with 10 ha of grassland/fodder maize/potato rotation and 23 dairy cows.

^jFarm with total area of 58 ha (27 ha of grassland, 13 ha of wheat and maize, 15 ha of forage maize, 2 ha of set aside) and 42 dairy cows.

^kFarm with 48 ha of arable cropland, 42 dairy cows, 15 heifers, and 40–60 beef cattle.

^lMainly due to changes in stocks of fodder and slurry between two consecutive years.

Table V
Phytic Acid (Inositol Hexaphosphate) Content in Some Feedstuffs^a

Feedstuff	Total P (%)	Phytic acid (%)	Phytic acid as % of total P
Grain sorghum ^a	0.31	0.21	68
Barley ^a	0.38	0.25	66
Soybean meal ^a	0.61	0.37	61
Normal corn ^b	0.27	0.19	70
Low phytic acid corn ^b	0.27	0.10	37

^aAdapted from Lott *et al.* (2000).

^bAdapted from Raboy *et al.* (2000) Raboy and Gerbasi (1996).

Addition of mineral P (calcium phosphates) is necessary in the diets of nonruminants (poultry, swine) because these animals lack the inherent phytase enzyme in the digestive tract (Bedford, 2000) and cannot digest phytic acid present in the diets. The majority of organic P (61–70%) in feed grains, such as maize, sorghum, and soybean meal, which are commonly fed to poultry and swine consist of phytic acid (Table V) (Lott, 1984; Nelson *et al.*, 1968). Phytic acid may bind with different minerals, such as Ca, Fe, Zn (Sandberg *et al.*, 1993), and proteins (Thompson, 1993), in the animal digestive tract due to presence of strong negative charges on its surface (Dao, 2003). This may result in reduced uptake of these minerals thereby causing nutrient deficiency problems such as anemia and osteoporosis (Oatway *et al.*, 2001). The development of corn cultivars with lower concentrations of phytic acid should help to increase utilization of feed P by animals and may provide another alternative to reduce P excretion in manures. For example, Raboy *et al.* (1984, 2000) and Raboy and Gerbasi (1996) have developed low phytic acid corn that has similar total P levels to nonmutant hybrids but lower levels of phytic acid (37% of total P) and higher levels of inorganic P (63% of total P) (Table V).

Klopfenstein *et al.* (2002) suggested that using the latest advances in diet management, such as adding phytase, feeding closer to animal requirement, using higher bioavailability feed ingredients, adding vitamin D₃ metabolites, and choosing low phytic acid ingredients could result in a 40% reduction in total P in poultry waste. Studies by Angel *et al.* (2005), Applegate *et al.* (2003), Maguire *et al.* (2004), and Toor *et al.* (2005c) have confirmed these observations that feeding P to requirement, including high available P corn (or low phytic acid corn) in diets, and supplementing diets with phytase can reduce total P excretion in poultry litters and swine manures by 40%. Similarly, P excretion in finished pigs manure can be reduced by 30–40% with dietary P management (Baxter *et al.*, 2003; Pierce *et al.*, 1997). Overall, these studies have concluded that the reduction of P overfeeding and

supplementation of phytase is a sound management practice that should be recommended to reduce total P excretion in the manures.

2. Dairy Diets

Excess P is often added to dairy diets due to the common supposition that P helps to maintain a better reproduction rate. This belief is largely based on a study conducted by Hignett and Hignett (1951) in the United Kingdom, where diets contained 0.10–0.25% P prior to P supplementation, however, most present day dairy diets contain greater than 0.30% P without P supplementation and sometimes as high as 0.40 to 0.45% P. The National Research Council's current recommendation (National Research Council, 2001) as well as a number of other studies (Dou *et al.*, 2002, 2003; Karn, 2001; Valk *et al.*, 2000; Wu *et al.*, 2001) propose that the P content in diets can be safely reduced to between 0.32 and 0.38%. These studies have shown that reduction of P in dairy diets will lead to reductions in P in manures. Dou *et al.* (2002) analyzed fecal samples and reported that increasing dietary P levels led to a higher concentration of total P in feces. Toor *et al.* (2005d) sampled 40 dairy farms in Mid-Atlantic United States and calculated that there will be about 40% more P to manage each year on dairy farms using high-P diets (mean of 21 farms: 5.1 g total dietary P kg⁻¹) than on farms feeding low-P diets (mean of 19 dairy farms: 3.6 g total dietary P kg⁻¹).

In summary, the modification of dairy and poultry diets by reducing P concentration in diets and adding phytase and using low phytic acid foodstuffs in poultry and swine diets can result in manures with approximately 40% less total P. Therefore, with the advent of the new century, we have new feeding management strategies for dairy, poultry, and swine that may result in manures with different chemical composition.

B. ORGANIC WASTES HANDLING EFFECTS

Prior to land application or other off-farm usage of organic wastes, they must be removed from animal houses and municipal treatment plants and transported to a storage facility or directly spread on land. Major waste handling and treatment factors that influence the amount and forms of P in manures are: type and amount of bedding material (e.g., straw, saw dust, wood shavings, paper and sand, nonlegume hay, alfalfa), addition of feed additives (e.g., phytase enzyme in poultry and swine diets) and manure amendments (e.g., aluminum sulfate in poultry litter), manure accumulation time, amount of water used to flush the house (e.g., in dairy parlors, typically 145–300 liter water is used per cow per day), and storage time prior to land application.

The following sections describe the major treatment and storage practices used today.

1. Treatments

Organic waste treatments consist of physical and chemical to biological processes, which are often needed to reduce the volumes of waste, destroy pathogens, control odors, and improve palatability. These treatments can also modify the physical, chemical, and biological characteristics of wastes, resulting in heterogeneity in P speciation from one production site to another. The following section summarizes the common treatments.

a. Physical Treatment Physical treatment of wastes involves solid-liquid separation by sedimentation or screening and is mostly used for dairy and swine manures and biosolids. The separated solids can then be composted, reused as bedding, or feed, or pelletized, or directly applied to soil as an amendment. The liquids can be used to flush the animal house to remove manure before using to supply plant nutrients. The major advantage of this process is lower transport costs due to reduction in waste volume, which can be of significant concern in large animal production facilities. Other alternative methods of manure use, primarily for poultry litter and biosolids, include drying, incineration, and pyrolysis. A detailed discussion of physical treatment processes for wastes has been provided by Moore (1993) and Day and Funk (1998).

b. Chemical Treatment Organic wastes can be treated with chemicals to precipitate particulates and colloidal matter, to control pH and odor, and to enhance biological treatment. The most commonly used coagulants in wastewater are aluminum sulfate, ferric sulfate or chloride, and lime. Some polyelectrolytes and polymers have also been used in biosolids and manures (Dao and Daniel, 2002; Dao *et al.*, 2001). In poultry houses, sodium bisulfite is used for NH_3 control, litter acidification, and for pathogen reduction (*Salmonella* and *Campylobacter*) (Moore *et al.*, 1996; Yang *et al.*, 1998). Another common chemical additive used today in poultry houses is aluminum sulfate (alum), which reduces soluble inorganic P concentrations and NH_3 emissions (Moore *et al.*, 2000; Sims and Luka-McCafferty, 2002). Usage of alum and aluminum chloride to reduce soluble inorganic P in swine manure has also been documented (Smith *et al.*, 2001).

c. Biological Treatment Biological treatment can significantly reduce the solids content of waste by the action of biological organisms in the presence (aerobic) or absence (anaerobic) of oxygen, thus changing the physical and chemical properties of the waste. Wastes are composed of

liquid and solid phases; the solids can be volatile or nonvolatile. Most of the solids (75–80%) in fresh waste are organic in nature, which contain both biodegradable and nonbiodegradable components. Microorganisms act on the biodegradable component and convert organic C, O, and H to CO_2 and H_2O in aerobic treatment and to CH_4 and CO_2 in anaerobic treatment.

Composting is a form of biological decomposition of organic matter by aerobic thermophilic organisms (bacteria, fungi) that produces a stable humus-like material (Miller, 1991), which may then be used as a potting material and soil amendment. It is most suited for solid manures and can considerably reduce weight, volume, and odor. Recently, dead animal composting has become a common method to dispose of animal carcasses (Cabrera and Sims, 2000; Murphy, 1988). In this process, protein and carbohydrates are metabolized to CO_2 , H_2O , NH_3 , and microbial cells. The primary goals of these treatment steps are to promote easy separation of solids from liquids and to reduce waste volume while retaining all of the important nutrients (N, P, K).

2. Storage

Organic wastes can be stored for short- or long-term periods depending upon the facilities in a given farm or facility. While solid manures such as dairy and poultry are relatively easy to handle and may be composted prior to land application, liquid manures such as swine manure and dairy effluent require additional storage structures such as concrete tanks or pits. Alternative low cost storage can be accomplished by storing liquid wastes in earthen ponds or lagoons. From these structures, liquid manure may be either directly pumped or carried in tanks for land application. The length and type of facility for storing the waste can have a profound influence on the forms of P, yet this important area of P research remains relatively unexplored. Toor *et al.* (2005a) found that inorganic P was increased by approximately 10% of total P by storing dairy feces in slurry pits due to microbial decomposition of organic forms of P such as phytic acid, DNA, and phospholipids. McGrath (2004) evaluated the effect of poultry litter moisture content during storage and reported that organic P was degraded to inorganic P. This degradation increased the concentration and percentage of WEP in litter, suggesting that the length of storage can significantly transform P species in resulting manures. On the other hand, Baxter *et al.* (2003) studied the effects of pig diets formulated with low phytic acid corn and phytase on P concentrations in pig slurry stored up to 150 days. They observed that the relative proportion of dissolved inorganic P as percent of total P in slurry decreased with increase in storage time from 0 to 150 days because of microbial assimilation of inorganic P or formation of less soluble P compounds.

IV. METHODS FOR CHARACTERIZING PHOSPHORUS IN ORGANIC WASTES

Knowledge of the total amounts of P in organic wastes may help to determine the amount of supplemental mineral P fertilizer that must be added for optimum crop yields, and can aid in P mass balance calculations for farms and watersheds. However, total P measurements provide no information about the nature of chemical forms of P present in wastes, which will be essential to understand fate and mobility of waste applied P. To partition total P into more specific chemical P forms in organic wastes, a number of methods, ranging from wet chemical and biological to spectroscopic have been employed and are discussed in the following sections.

A. TOTAL PHOSPHORUS

Total P analysis of organic wastes involves conversion of insoluble components into soluble forms by some form of digestion followed by P determination in the solution. Digestion methods for wastes are principally adapted from plant digestion methods as wastes contain digested plant and feed components. A number of oxidizing agents, such as H_2SO_4 , HNO_3 , or HClO_4 and H_2O_2 , can be used. Detailed descriptions of the methods that involve the use of (i) HNO_3 and HClO_4 , (ii) H_2SO_4 and HNO_3 , and (iii) H_2SO_4 and $(\text{NH}_4)_2\text{S}_2\text{O}_8$ or $\text{K}_2\text{S}_2\text{O}_8$ are given by APHA (1989). Other workers have used the H_2SO_4 – H_2O_2 –HF digestion (Bowman, 1988), Na_2CO_3 fusion (Olsen and Sommers, 1982), NaOBr – NaOH oxidation (Dick and Tabatabai, 1977), and ignition (Saunders and Williams, 1955) methods for determining total P in soils. All these methods give comparable results but may require special equipment such as fume hoods for HClO_4 digestion, expensive platinum crucibles for the Na_2CO_3 fusion method, and HF resistant glass material for the H_2SO_4 – H_2O_2 –HF digestion method. The US Environmental Protection Agency's (1986) method uses HNO_3 followed by H_2O_2 , commonly referred as EPA 3050 and EPA3051, and has been the most widely used method for digestion of manures and biosolids to determine total P in the United States.

After digestion of wastes, P in solution can be measured colorimetrically by the acid molybdate (Murphy and Riley, 1962) or by the ICP-OES method. The acid molybdate method was originally developed by Osmond (1887) but became more widely used following modification by Murphy and Riley (1962). Both acid molybdate and ICP give similar results for total P analysis. However, the main difficulty can arise during analysis of inorganic P with acid molybdate in undigested samples (extracted with water or

Table VI
Effect of Method of Determination on Water Extractable Phosphorus in Poultry Litter^a

Manure from diet	Water soluble P		
	Colorimetry ^b (mg kg ⁻¹)	ICP-OES ^c (mg kg ⁻¹)	Overestimation with ICP-OES (%)
Normal corn (NORC)	2394	2486	4
High available P corn (HAP)	2349	2525	7
NORC-0.1% NPP + phytase ^d	2157	2395	10
HAPC-0.1% NPP + phytase ^e	1235	1414	13
NORC-0.2% NPP + phytase ^f	937	1305	28
HAPC-0.2% NPP + phytase ^g	418	541	23
LSD _{0.05}	351	235	

^aAdapted from Sims *et al.* (2000).

^bExtraction of “as-is” sample at 1:10 ratio (for 1 h), filtration through Whatman #4 and 0.45 µm filters, P measured colorimetrically.

^cExtraction of “as-is” sample at 1:10 ratio (for 1 h), filtration through Whatman #4 and 0.45 µm filters, P measured by ICP-OES.

^dNormal corn with 0.1% less nonphytate P (NPP) and phytase.

^eHigh available P corn with 0.1% less NPP and phytase.

^fNormal corn with 0.2% less NPP and phytase.

^gHigh available P corn with 0.2% less NPP and phytase.

Phytase was added at 650 U kg⁻¹.

dilute acids) as sample acidification can result in hydrolysis of acid labile organic compounds, such as sugar phosphates and monophosphate esters (McKelvie *et al.*, 1995), and cause inflated inorganic P values. On the other hand, ICP measures both inorganic and all organic P. For example, in poultry litter generated from modified diets, Sims *et al.* (2000) observed 4–28% higher WEP measured by the ICP than the molybdate method (Table VI).

Another approach to rapidly analyze total P in manures is near-infrared reflectance spectroscopy (NIRS). The principle behind NIRS and information on its application in agricultural and food products can be found in Burns and Cziurczak (1992) and Williams and Norris (2001). Although total C and N concentrations have been successfully analyzed in manures by NIRS, there have been mixed results when quantifying the total P content in manures with NIRS: some workers obtained good correlation between the total P measured with NIRS and total chemical analyses (Malley *et al.*, 2002) while others did not (Reeves, 2001; Reeves and Van Kessel, 2000). If this method can be standardized to determine total P in wastes, it has the potential to become a robust, cost-effective means for use in routine analyses by manure testing laboratories and *in situ* field monitoring.

B. WATER EXTRACTABLE PHOSPHORUS

Water extractable P is that fraction of total P, which is extracted by shaking a known amount of waste with water. There has been a growing interest in the use of WEP as an indicator to assess the relative proportion of dissolved P in organic wastes that could be subject to loss on land application. Studies have shown that WEP can range from <10 to as high as 75% of total P in biosolids (Brandt *et al.*, 2004; Huang and Shenker, 2004), dairy, poultry, and swine manures (Angel *et al.*, 2005; Applegate *et al.*, 2003; Baxter *et al.*, 2003; Dou *et al.*, 2002; Kleinman *et al.*, 2005; Maguire *et al.*, 2003; Toor *et al.*, 2005c). A positive correlation between WEP in wastes and dissolved P in surface runoff and leachate has been reported (Kleinman *et al.*, 2002; McDowell and Sharpley, 2001), suggesting its applicability for environmental purposes. In addition, it has also been proposed that WEP or other dilute extractants can be used to provide an *in situ* management tool to diagnose excessive P feeding and to assess the relative P availability in waste. This use of WEP testing as a fecal P indicator would improve optimization of P efficiency in the confined animal operations (Dou *et al.*, 2002).

Water extractable P consists of organic and inorganic forms of P. Inorganic P is largely composed of dissolved inorganic P or dissolved P minerals. For example, Toor *et al.* (2005c) observed a significant correlation between WEP and dicalcium phosphate ($r = 0.85$) in poultry litters generated from modified diets. Information about the exact nature of organic P species in water extracts of wastes is limited at this time, but it is likely that WEP contains a mixture of organic P species such as labile monoester P and diester P (McKelvie *et al.*, 1995).

When comparing WEP results from different literature sources using manure and biosolids, the factors discussed in the following section, such as waste to solution ratio, length of shaking, fresh or dry samples, centrifugation or filtration, and method of analysis (discussed in total P section), should be kept in mind as they exert a major influence on measured WEP.

Waste to solution ratio can affect the amount of WEP in organic wastes. Various researchers have used different waste to water extraction ratios and have referred to this fraction as WEP. This has made comparisons between different studies rather difficult. For example, Toor and Sims (unpublished data) observed an increase in concentrations of WEP (inorganic and organic) in dairy feces with increase in manure to solution ratio from 1:10 to 1:400 (Fig. 1). Standardization of WEP methodology has occurred (Kleinman *et al.*, 2002, 2005; Wolf *et al.*, 2005) and many researchers now use a waste to solution ratio of 1:100.

Length of shaking waste with extracting solution can also affect the amount of WEP extracted. However, the temporal effect is primarily dependent upon the waste to solution ratio. At a large waste to solution ratio (e.g.,

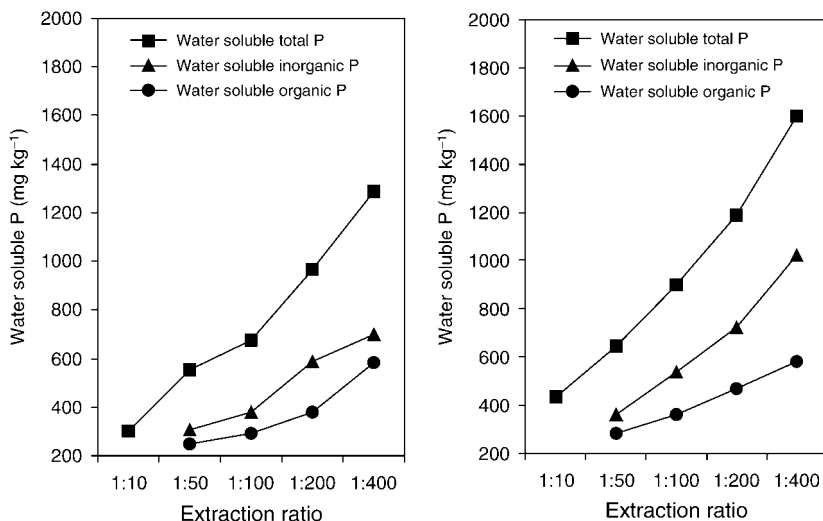


Figure 1 Effect of extraction ratio on water soluble phosphorus in two dairy feces samples collected from Mid-Atlantic United States.

>1:100), more P may be released into the solution after a longer shaking time. The chemical composition of the wastes is the principal factor affecting the release of P. If there is a higher pool of soluble P in the waste then the release of P may continue at longer extraction times.

The amount of WEP in organic wastes can also be affected by drying the samples. Ajiboye *et al.* (2004) compared WEP in fresh and oven-dried (105°C) organic wastes (dairy, hog manures) and noted an increase in WEP (Fig. 2). They attributed this increase to hydrolysis of water extractable organic P to inorganic P in hog manures, and both transformation of NaHCO_3 inorganic P and hydrolysis of NaHCO_3 organic P to water extractable inorganic P in dairy manures. In general, drying samples can kill microorganisms resulting in the release of cellular P, or, can cause desorption of P held on the surfaces of organic and inorganic colloids, thus, increasing the WEP of the materials.

After extractions, separation of waste and water suspension is accomplished via either filtration or centrifugation. It is assumed that filtration will remove the P associated with particulates, whereas centrifugation will not easily remove this particulate fraction. Sims *et al.* (2000) found that WEP was 62–78% higher in centrifuged (no filtration) poultry litter extracts than those filtered through 0.45- μm filter paper (Table VII).

Although there remains some heterogeneity in WEP measurement introduced from sample processing (fresh or dry samples) and analysis (waste to solution ratio, length of shaking, centrifugation, or filtration), WEP is an

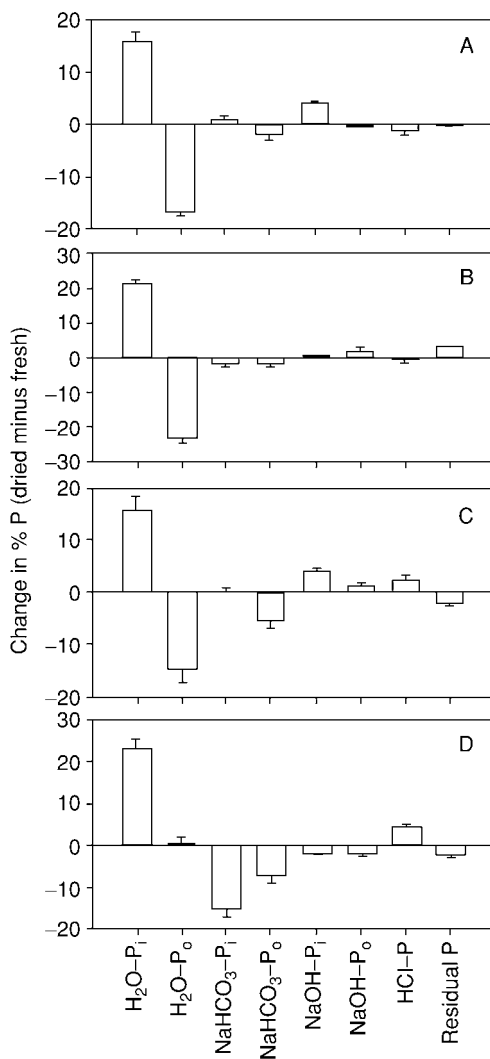


Figure 2 Transformation of phosphorus fractions with oven-drying of the amendments. (A) Hog manure collected from an agitated storage lagoon, (B) hog manure from a sow barn (C) hog manure from a nursery barn, and (D) manure from a dairy barn. The whiskers represent standard errors of the means of three replicates. The terms H₂O-P_i and H₂O-P_o are water-extractable inorganic and organic P, NaHCO₃-P_i and NaHCO₃-P_o are NaHCO₃-extractable inorganic and organic P, NaOH-P_i and NaOH-P_o are NaOH-extractable inorganic and organic P; and HCl-P is HCl-extractable P. After Ajiboye *et al.* (2004).

Table VII
Effect of Filtration Versus Centrifugation on Water Extractable Phosphorus Release
from Poultry Litter^a

Manure from diet	Water soluble P		
	Filtration ^b (mg kg ⁻¹)	Centrifugation (no filtration) ^c (mg kg ⁻¹)	Overestimation with centrifugation (%)
Normal corn (NORC)	2486	6800	63
High available P corn (HAP)	2525	6624	62
NORC-0.1% NPP + phytase ^d	2395	7045	66
HAPC-0.1% NPP + phytase ^e	1414	5030	72
NORC-0.2% NPP + phytase ^f	1305	3561	63
HAPC-0.2% NPP + phytase ^g	541	2497	78
LSD _{0.05}	235	1215	

^aAdapted from Sims *et al.* (2000).

^bExtraction of “as-is” sample at 1:10 ratio (for 1 h), filtration through Whatman #4 and 0.45 µm filters, P measured colorimetrically.

^cExtraction of “as-is” sample at 1:10 ratio (for 1 h), filtration through Whatman #4 and 0.45 µm filters, P measured by ICP-AES.

^dNormal corn with 0.1% less nonphytate P (NPP) and phytase.

^eHigh available P corn with 0.1% less NPP and phytase.

^fNormal corn with 0.2% less NPP and phytase.

^gHigh available P corn with 0.2% less NPP and phytase.

Phytase was added at 650 U kg⁻¹.

important part of total P that should be measured in wastes. WEP results are extremely useful in understanding both the plant available P pool and the resulting potential influence on water quality if these wastes were to reach water bodies.

C. PHYSICOCHEMICAL FRACTIONATION

The physicochemical fractionation method is widely used to differentiate P forms in leachate and surface runoff waters (Haygarth and Sharpley, 2000), and this method can also be used to characterize P forms in liquid wastes such as dairy and swine slurry and wastewater produced from sewage treatment. Physicochemical fractionation of P in liquid wastes into reactive, unreactive, dissolved, and particulate forms (Fig. 3) is based on two simple criteria: (1) size: whether the P is present in the dissolved or particulate fraction, with the distinction made by filtration through a filter paper (usually 0.45 or 0.2 µm); and (2) digestibility: whether the P is present in a reactive or unreactive fraction, with the distinction made by digesting the wastes with some form of acid or oxidizing agent (Haygarth and Sharpley,

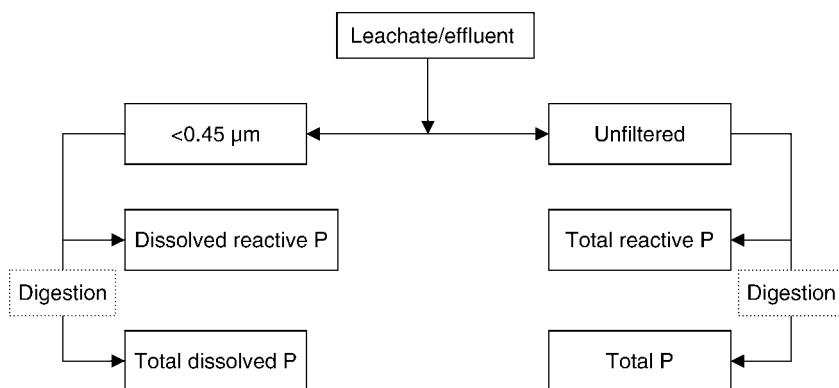


Figure 3 Physicochemical fractionation of phosphorus.

2000). Dissolved reactive P and total reactive P are directly measured (without digestion) on filtered and unfiltered samples, respectively, by the acid molybdate method. Both total dissolved P and total P fractions are determined on filtered and unfiltered samples, respectively, after digestion (Ebina *et al.*, 1983; Rowland and Haygarth, 1997). The other P forms are calculated as:

- Total particulate P = total P – total dissolved P
- Total unreactive P = total P – total reactive P
- Dissolved unreactive P = total dissolved P – dissolved reactive P
- Particulate unreactive P = total unreactive P – dissolved unreactive P
- Particulate reactive P = total reactive P – dissolved reactive P.

According to this fractionation scheme, there are four categories of P forms under two main groups. Reactive P is thought to mainly consist of orthophosphate and includes P in dissolved and particulate phases. Unreactive dissolved and particulate P contains labile and recalcitrant organic P compounds. The possible composition of P compounds in these physicochemical forms is as follows:

Dissolved reactive P includes orthophosphate that are soluble in water and some of the easily hydrolyzable organic P forms such as labile monoesters (sugar phosphates, mononucleotides) and diesters (DNA, RNA, phospholipids). The organic P species in the fraction are a result of the acid-mediated hydrolysis during P analysis by acid molybdate method (McKelvie *et al.*, 1995).

Particulate reactive P is that fraction of total P that is insoluble in water but may contain P sorbed on the surfaces of clay, Fe, Al, or Ca oxides and hydroxides.

Dissolved unreactive P is thought to primarily contain organic P compounds, however, this fraction can also contain some inorganic compounds, such as

Table VIII
Composition of Dissolved Unreactive Phosphorus in Marine Environments^a

Dissolved unreactive P compound class	Composition (%)	Method of analysis
Monophosphate esters	10–100	Enzymatic assays
	55–77	Modified UV oxidation
Nucleotides and nucleic acids	23–5	Persulfate-modified UV
	10–100	Enzymatic assays
	ATP <1	Firefly bioluminescence
	DNA/RNA <5	Multiple methods
Phospholipids	3–11	Cross flow filtration and Polymyxin B treatment
Phosphonates	5–10	³¹ P-NMR
Polyphosphates	0–50	Acid reflux–UV oxidation

^aAdapted from Benitez-Nelson (2000).

polyphosphates, which are not detected with the acid molybdate method (Ron Vaz *et al.*, 1993; Thomson-Bulldis and Karl, 1998). Relatively less information is available about the nature of organic P forms in this pool, whereas some data are available on the nature of organic P compounds in marine waters and sediments. Benitez-Nelson (2000) using various methods such as enzymatic assays, ultraviolet (UV) oxidation, cross flow filtration, and ³¹P-NMR distinguished various classes of organic P compounds in marine waters and sediments into monophosphate esters, nucleotides and nucleic acid, phospholipids, phosphonates, and polyphosphates (Table VIII).

Particulate unreactive P is a fraction in which the nature of the P compounds is relatively unknown. However, it may contain P sorbed on mineral–humic acid complexes. Toor *et al.* (2005b) speculated that particulate unreactive P in dairy effluent may originate from coatings of soil particles (e.g., clay, Ca, Fe, Al), eaten by dairy cows during grazing in animal rumen, by inositol hexaphosphate.

Adequate knowledge about P speciation in wastes, gained by physicochemical fractionation, can provide invaluable information about the potential bioavailability of these P forms if they reach water bodies. For example, dissolved reactive P is known to be immediately available to aquatic biota, while dissolved unreactive P may become available over a short period of time (Whitton *et al.*, 1991). On the other hand, the recalcitrant nature of particulate reactive and unreactive P in the environment means that these fractions have a low bioaccessibility and might not constitute a direct short-term threat to surface water quality. Therefore, physicochemical fractionation can provide a rapid and low cost means to assess the relative bioavailability of different forms of P in organic wastes.

D. SEQUENTIAL PHOSPHORUS FRACTIONATION

Sequential chemical P fractionation is primarily used for solid materials but can also be used for liquid wastes after some form of drying (e.g., air-, oven-, freeze-drying). The earliest work on P fractionation in manures using chemical extractants was documented by Funatsu (1908) and Tsuda (1909). They sequentially extracted P forms in herring guano as: (i) acid soluble (inorganic P, phytic acid type material), (ii) alcohol-ether soluble (phospholipids), and (iii) residual (nucleic acid type material). Other workers later used this methodology to separate P forms in poultry manure, farmyard manure, and cow manure (McAuliffe and Peech, 1949). They found that organic and inorganic P was present in equal proportions. This fractionation scheme was later adopted by Peperzak *et al.* (1959) and later Barnett (1994a,b) modified this method, as illustrated in Fig. 4, and analyzed a large number of dairy, poultry, and hog manures (Table IX). However, this method is not widely used by many researchers because it provides limited information about organic P forms, does not distinguish between inorganic P forms, and is time consuming.

The other sequential chemical fractionation method, which has been more popular among researchers, is largely adopted from the fractionation method suggested for soils by Hedley *et al.* (1982b). The Hedley fractionation scheme employs acid or alkaline reagents (mild to strong), where material (soil, waste) is sequentially extracted with H₂O or resin, NaHCO₃, NaOH, HCl, or H₂SO₄ and then the P extracted by each extractant is analyzed for inorganic P and total P (Fig. 5). The difference between the total P and inorganic P in each extractant is assumed to be organic P.

For soils, H₂O or resin extractable P is thought to be composed of dissolved inorganic P, whereas NaHCO₃ and NaOH extractable fractions may be a mixture of amorphous and crystalline Al and Fe phosphates, and some physically and chemically protected organic P. The relatively stable fractions extracted with acids (HCl or H₂SO₄) are assumed to be Ca bound phosphates. Use of this approach often assumes that similar forms exist in wastes although the matrices of manures are based on organic residues while soils are dominated by mineral phases. In addition, soils contain more Al and Fe than manures, while manures have greater concentrations of Ca and Mg than soils.

Leinweber *et al.* (1997) and Sharpley and Moyer (2000) and Dou *et al.* (2000) used the Hedley fractionation method to characterize P in a range of dairy, poultry, and swine manures (Table X). Dou *et al.* (2000) reported that H₂O, NaHCO₃, NaOH, and HCl removed 67, 13, 5, and 5% P for dairy manures and 50, 20, 5, and 25% P for poultry manures, respectively. The major amount of P was extracted from manures with H₂O and NaHCO₃. This is in contrast with soils, where most of the P was removed with NaOH

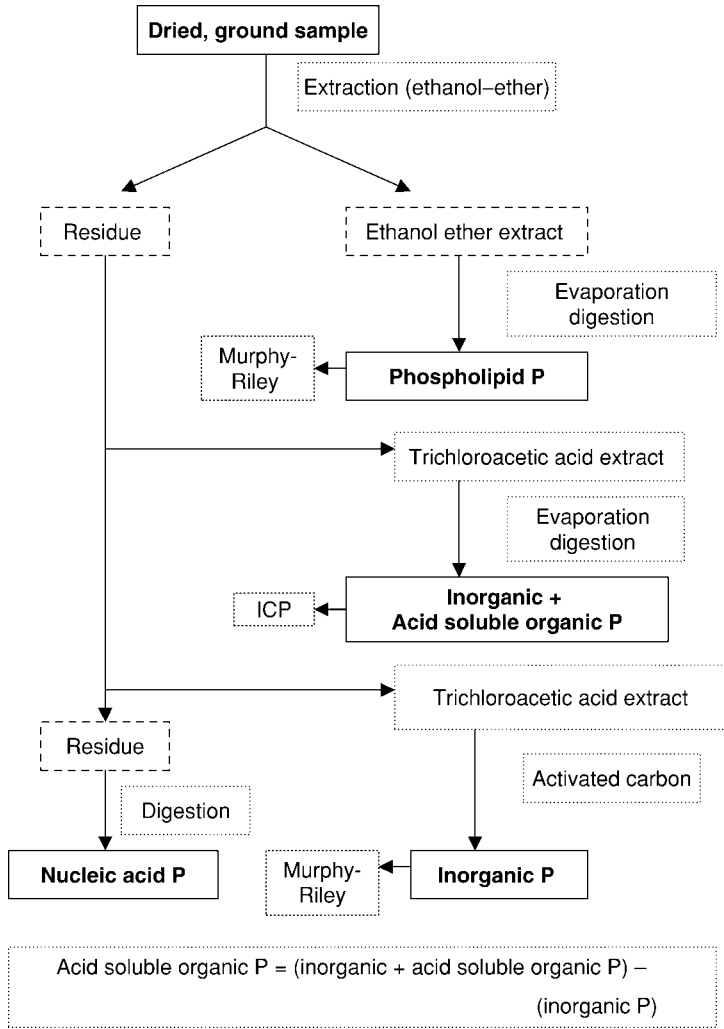


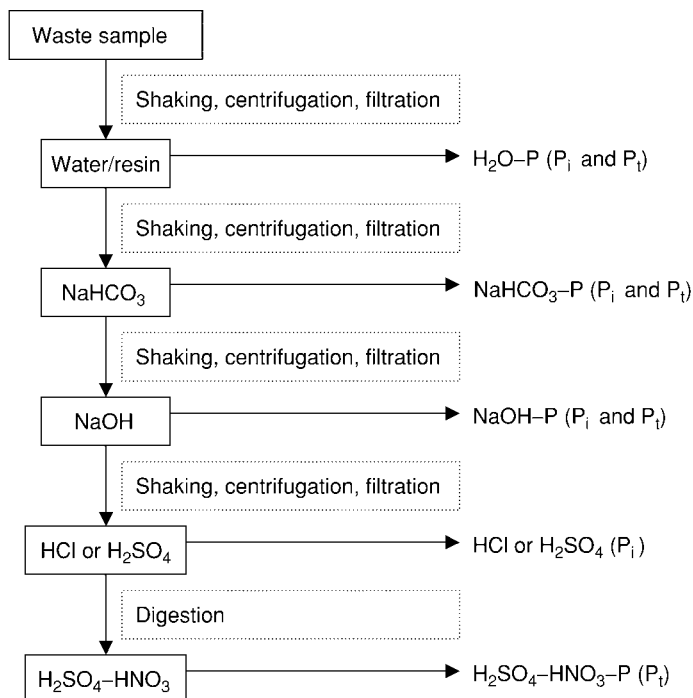
Figure 4 Manure phosphorus fractionation scheme. Adapted from Barnett (1994a).

and HCl extractions (He *et al.*, 2003a; Sharpley, 1996; Tiessen *et al.*, 1984). Therefore, it is inappropriate to apply soil fractionation procedures on manures, as it is not clear exactly what forms of P are being removed by the different fractionation steps. However, with precise knowledge of extracted P forms available via spectroscopic methods, as discussed in the following section, it should be possible to accurately develop and interpret waste fractionation procedures.

Table IX
Fractionation of Phosphorus Forms in Various Manures According to the Fractionation Scheme of Barnett (1994b) as Shown in Fig. 4^a

Manure type	Total P (g kg ⁻¹)		Percentage of total P	Range (%)	Coefficient of variation (%)
Dairy (15 herds)	9 (6–16) ^b	Inorganic P	63.2	55.3–71.2	8.1
		Residual P ^c	27.7	18.1–35.8	18.4
		Acid soluble organic P ^d	7.8	2.4–13.9	46.7
		Lipid P ^e	1.4	1.1–1.8	19.6
Broiler (13 flocks)	18 (13–23)	Inorganic P	34.8	21.4–58.4	31.1
		Residual P	11	1.5–16.6	45.7
		Acid soluble organic P	53.4	26.2–75.5	24.4
		Lipid P	0.9	0.4–1.3	33.4
Layer (11 flocks)	24 (16–30)	Inorganic P	49.3	39.8–70.0	21.4
		Residual P	17.3	6.6–31.8	41.2
		Acid soluble organic P	33.2	24.7–44.4	20.2
		Lipid P	0.6	0.4–0.8	19.5
Hogs (16 herds)	29 (20–40)	Inorganic P	54.7	42.2–76.6	20.3
		Residual P	15.2	9.2–26.9	36
		Acid soluble organic P	29.7	13.7–45.3	41.6
		Lipid P	0.4	0.3–0.5	15.8

^aAdapted from Barnett (1994b).
^bRange of values.
^cNucleic acid type material.
^dInositol hexaphosphate or phytic acid type material.
^ePhospholipids.



P_i = inorganic P determined by Murphy-Riley.

P_t = total P determined by digestion followed by Murphy-Riley or ICP-OES.

P_o = organic P (calculated as the difference between total P and inorganic P at each step).

Figure 5 Sequential waste phosphorus fractionation scheme. Adapted from Hedley *et al.* (1982b) and Tiessen *et al.* (1984).

Case Study: Identification of Phosphorus Compounds in Cattle, Broiler, and Swine Manure

Turner and Leytem (2004) first sequentially extracted P forms from broiler, cattle, and swine manures and then performed solution state NMR analysis on these extracts. Their results indicated that H_2O extraction primarily removed orthophosphate (>89% of total P) in swine manure and broiler litter, while the H_2O extract of cattle manure contained most of the P as organic forms (monoesters: 37%, DNA: 20%) and only 42% as orthophosphate (Table XI). Orthophosphate was 82–98% in $NaHCO_3$ extracts of cattle and swine manure, whereas broiler litter $NaHCO_3$ extracts contained

Table X
Phosphorus Fractions in Manures and Composts according to the Fractionation Scheme of Sharpley and Moyer (2000) as Shown in Fig. 5^a

	Dairy manure	Dairy compost	Poultry manure	Poultry litter	Poultry compost	Swine slurry
Dry matter (%)	33	37	39	90	68	10
Total P (mg kg ⁻¹)	3990	16,530	28,650	16,320	9440	32,950
C to P ratio	87	20	13	22	13	8
Inorganic P (%) ^b						
H ₂ O	51	15	26	25	21	18
NaHCO ₃	9	36	25	31	31	13
NaOH	2	8	1	5	9	50
HCl	1	33	32	29	27	10
Total	63	92	84	90	87	91
Organic P (%) ^b						
H ₂ O	12	1	8	3	1	5
NaHCO ₃	2	1	4	4	8	2
NaOH	11	3	2	2	2	1
Total (%)	25	5	14	9	11	8
Residual P (%) ^b	12	3	2	1	1	1

^aAdapted from Sharpley and Moyer (2000).

^bPercentage of the total P concentration of manure or compost.

18% monoesters. The NaOH extracts of cattle and swine manures contained 52–59% orthophosphate with the remainder as monoesters (37–38%) and pyrophosphates (3–12%). Similarly, orthophosphate in the HCl extracts of manures ranged from 14% (broiler litter) to 66% (swine manure) and 100% (cattle manure). On the other hand, most P (86%) was present as phytic acid in both NaOH and HCl extracts of broiler litter. Overall, the use of NMR spectroscopy on sequential fractions of manures has greatly improved our knowledge of various inorganic and organic P compounds extracted by different reagents.

Generally, P extracted by mild extraction (H₂O, NaHCO₃) in the study by Turner and Leytem (2004) was in more soluble P forms, such as orthophosphate, phospholipids, DNA, and labile monoesters, whereas the P compounds extracted by strong reagents (NaOH, HCl) contained orthophosphate and phytic acid. Although this study provided a wealth of information about organic P compounds present in the different extracts of manures, one of the limitations with the use of solution state NMR spectroscopy is the inability to characterize inorganic forms of P orthophosphate into various pools such as more soluble (dicalcium phosphate) to less soluble (apatite). For example, it would be difficult to know if the orthophosphate extracted by NaHCO₃ or HCl are from dicalcium phosphate or apatite

Table XI
Concentrations of Phosphorus Compounds (in mg P kg⁻¹ dry weight) in Sequential Extracts of Animal Manures From the Hedley Fractionation Procedure Determined by Solution ³¹P-NMR Spectroscopy and ICP-OES Spectrometry^a

	Total phosphorus	Phosphate ^b	Phosphate monoesters ^c	Phospho lipids ^c	DNA ^c	Pyrophosphate ^c
Broiler litter						
Water	4,547 (29)	4036 (89)	291 (6)	138 (3)	82 (2)	ND
NaHCO ₃	826 (5)	679 (82)	147 (18)	ND ^d	ND	ND
NaOH	1,854 (12)	266 (14)	1588 (86) ^e	ND	ND	Tr ^f
HCl	7,734 (48)	1114 (14)	6620 (86) ^e	ND	ND	ND
Sum of fractions	14,961 (94)	6095 (41)	8647 (58)	138 (<1)	82 (<1)	Tr
Cattle manure						
Water	537 (11)	228 (42)	200 (37)	ND	109 (20)	ND
NaHCO ₃	2,116 (43)	2077 (98)	Tr	ND	ND	39 (2)
NaOH	952 (33)	492 (52)	350 (37)	ND	ND	110 (12)
HCl	311 (6)	311 (100)	ND	ND	Nd	ND
Sum of fractions	3,916 (79)	3107 (79)	550 (14)	ND	109 (3)	150 (4)
Swine manure						
278 (3)	7,992 (55)	7644 (96)		70 (<1)	Tr	Tr
NaHCO ₃	3,419 (23)	3308 (97)	111 (3)	ND	ND	Nd
NaOH	846 (6)	498 (59)	325 (38) ^g	ND	ND	24 (3)
HCl	1,252 (9)	830 (66)	422 (34) ^h	ND	ND	ND
Sum of fractions	13,508 (92)	12,279 (91)	1136 (8)	70 (<1)	Tr	24 (<1)

^aAdapted from Turner and Leytem (2004).

^bData were determined by ICP-OES and are the mean of three replicate extracts. Values in parentheses are the recovery (%) of the total manure phosphorus in each extract.

^cDetermined by solution ³¹P-NMR spectroscopy. Values in parentheses are the proportion (%) of the total phosphorus in each extract.

^dND, not detected.

^eAll phosphate monoesters were phytic acid (calculated by sum of signals).

^fTr, trace.

^gPhytic acid concentration (C2*6) was 118 mg P kg⁻¹ dry wt (14% of the extracted P).

^hPhytic acid concentration (sum of signals) was 401 mg P kg⁻¹ dry wt (32% of the extracted P).

mineral although it could be assumed that HCl probably extracts less soluble forms of P (apatite). The understanding of orthophosphate in these fractions can be greatly improved by mineral identification in the sequential extracts. Toor *et al.* (2005c) used XANES spectroscopy and sequential chemical fractionation as methods to understand P compounds in poultry litters and turkey manures generated from modified diets. Their results demonstrated that H₂O extraction not only removed the dissolved inorganic P present in the solution phase but also extracted P from dicalcium phosphate mineral. In contrast, HCl extraction removed phytic acid from poultry litters and a mixture of phytic acid and hydroxylapatite from turkey manures.

The presence of all the P as orthophosphate in the HCl extract of cattle manure and 86% of P as phytic acid in broiler litter in Turner and Leytem's study (2004) suggests that HCl extraction removed Ca-phosphate minerals from cattle manure and phytic acid from broiler litter. This contention is supported by Toor *et al.* (2005c), where total Ca to P ratio of >2 in turkey manures resulted in formation of a less soluble P mineral (hydroxylapatite), while Ca to P ratio of <2 favored formation of a more soluble P mineral (dicalcium phosphate). The results of Toor *et al.* (2005c) can be used to understand the nature of orthophosphate in the study by Turner and Leytem (2004). It is likely that the higher total Ca to P ratio of 3.2 in the cattle manure studied by Turner and Leytem (2004) resulted in formation of apatite—a part of which was extracted by HCl. On the other hand, the total Ca to P ratio was lower in the broiler litter (1.3) and swine manure (0.8), therefore, it is likely that most of the orthophosphate measured in the NaHCO_3 or HCl extracts of these manures was either present in solution or extracted from more soluble minerals, such as dicalcium phosphate.

In summary, it is economical and relatively easy to analyze a large number of waste samples using the Hedley fractionation method compared with more expensive and time consuming techniques such as NMR and XANES. But it is inadmissible to relate the P forms separated with sequential chemical extraction alone to actual P forms present in wastes. The combined use of chemical and spectroscopic methods can help us to better interpret chemical fractionation methods. The results from the above studies (Toor *et al.*, 2005c; Turner and Leytem, 2004) can aid in characterization of P compounds in sequential chemical extracts of organic wastes and help to better understand the fate of P forms after land application.

E. ENZYME HYDROLYSIS

Organic wastes are reported to contain up to 40% P in organic forms (Barnett, 1994a; Maguire *et al.*, 2004; Sharpley and Moyer, 2000; Toor *et al.*, 2005c). Organic wastes mainly contain four types of organic P compounds: inositol phosphates, sugar phosphates, nucleic acids, and phospholipids. The availability of these organic P forms to plants or in environment depends upon the ability of phosphatase enzymes to hydrolyze organic P forms to inorganic P. The major organic forms of P present in organic wastes are briefly discussed before the details about enzyme hydrolysis method.

Inositol phosphates are esters of hexahydroxycyclohexane and exist in various forms. The most common is myoinositol, although other forms such as neo-, scyllo-, and D-chiro-inositol phosphate also exist in nature. The salt

of myoinositol is referred to as myoinositol hexakisphosphoric acid or phytic acid. Using a chemical P fractionation scheme, Barnett (1994b) determined that phytic acid type material was 7.8% in dairy manure, 29.7% in hog feces, 33.2% in layer litter, and 53.4% of total P in broiler litter (Table IX). Studies with advanced analytical techniques, such as NMR and XANES spectroscopy, have shown that phytic acid in organic wastes (dairy, poultry, turkey) can range from 25 to 40% (Maguire *et al.*, 2004; Toor *et al.*, 2005a; Turner and Leytem, 2004), which is within the limits of the Barnett (1994b) study.

Nucleic acids are the major component of plants, found in all living organisms, and are excreted as microbial products in manures. Two known derivatives of nucleic acids are ribonucleic acid (RNA) and deoxyribonucleic acid (DNA), which occur as a chain of nucleotides. Barnett (1994b) reported that residual P type material (DNA, RNA) in a range of broiler, hog, layer, and dairy manures was 11, 15.2, 17.3, and 27.7% of total P. Studies with NMR spectroscopy indicated that nucleic acids (e.g., DNA) in animal manures can range from 0 to 5% (Toor *et al.*, 2005a; Turner, 2004). The higher percentage of residual P type material determined by Barnett (1994b) in manures may include some of the mineral P species, such as Ca phosphates, as P was measured in the residue by acid digestion.

Phospholipids are organic compounds that are insoluble in water but soluble in lipophilic solvents such as benzene, chloroform, or ether. Lecithin or phosphatidylcholine is the most predominant soil phospholipid (~40%) followed by phosphatidylethanolamine (~30%). Of the remaining phospholipids, phosphatidylinositol, and phosphatidylserine are present in small amounts. Phospholipids, together with nucleic acids, are the major forms of organic P in plant tissue (Bieleski, 1973), however, they are present in only small amounts in soils (<7%) due to their rapid degradation (Anderson, 1967). The concentrations of phospholipids in dairy, poultry, and swine manures have been reported to be less than 2% by extraction with a mixture of ethanol and diethyl ether (Barnett, 1994b; McAuliffe and Peech, 1949; Peperzak *et al.*, 1959) and up to 10% by NMR spectroscopy (Toor *et al.*, 2005a; Turner, 2004). In addition to the presence of these phospholipid species in soils and manures, these have also been characterized with high performance liquid chromatography (HPLC) into phosphatidylethanolamine and phosphatidylcholine in a range of municipal biosolids (Stott and Tabatabai, 1985).

An emerging approach to characterize these organic P forms in wastes involves the use of known commercial phosphatase enzymes, which hydrolyze different organic P compounds (Table XII). In this method, waste extract (extracted with H₂O or chemical extractants) is incubated with appropriate enzyme mixture at constant temperature and specific time.

Table XII
Phosphatases Used to Identify Organic Phosphorus Forms in Soil and Manure Extracts

Reference	Enzyme	EC No.	Type	Source	Function	Specified activity	Comment
He and Honeycut (2001), Pant and Warman (2000)	Acid phosphatase	3.1.3.2	Type I	Wheat germ	Release orthophosphate from R-PO ₄ (monoester)	0.8 units mg ⁻¹ solid	Contains lipase
He and Honeycut (2001)	Alkaline phosphatase	3.1.3.1	Type VII-S	Bovine intestinal mucosa	Release orthophosphate from R-PO ₄ (monoester)	One unit will hydrolyze 1μmol of 4-nitrophenol phosphate per min at pH 9.8 at 37°C	ns ^a
Pant and Warman (2000)	Alkaline phosphatase	3.1.3.1	Type XXX-A	Calf intestinal mucosa	Release orthophosphate from R-PO ₄ (monoester)	1000 units/0.09 cm ³	ns
He and Honeycut (2001), Turner <i>et al.</i> (2002), Toor <i>et al.</i> (2003)	Fungal phytase	3.1.3.8	Myoinositol hexakis-phosphate 3-phospho-hydrolase	<i>Aspergillus ficcum</i>	Release orthophosphate from phytate	3.5 units mg ⁻¹ solid (one unit will release 1 mmol of inorganic P min ⁻¹ from 4.2 mmol of inositol hexaphosphate at pH 2.5)	This crude phytase source was expected to also contain other phosphatase enzymes
Hayes <i>et al.</i> (2000)	Phytase	3.1.3.8	ns	<i>Aspergillus niger</i>	Release orthophosphate from phytate	1.5 units mg ⁻¹ solid. one unit will liberate 1 μmol of inorganic P from 4.2 × 10 ⁻² M myoinositol hexakis dihydrogen phosphate per min at pH 2.5 at 37°C	ns
He and Honeycut (2001)	Wheat phytase	3.1.3.26	ns	Wheat	Release orthophosphate from phytate	One unit will liberate 1 μmol of inorganic phosphorus from 1.5 × 10 ⁻³ phytate per min at pH 5.15 at 55°C	Crude source

He and Honeycut (2001)	Nucleotide pyrophosphatase	3.6.1.9	Type II	<i>Crotalus adamanteus</i> venom	Cleavage of dinucleotide bond	ns	ns
He and Honeycut (2001)	Nuclease P1	3.1.30.1	ns	<i>Penicillium citrinum</i>	Endonucleolytic cleavage of RNA and DNA	ns	ns
Turner <i>et al.</i> (2002), Toor <i>et al.</i> (2003)	Alkaline phosphatase	3.1.3.2	Type III chromatographically purified	<i>Escherichia coli</i>	ns	60 units mg ⁻¹ protein (3.6 mg protein ml ⁻¹). One unit will hydrolyze 1 µmol of 4-nitrophenyl phosphate per min at pH 10.4 at 37°C	This highly pure source does not contain contaminant enzymes found in crude sources such as those used by Shand and Smith (1997)
Turner <i>et al.</i> (2002), Toor <i>et al.</i> (2003)	Phospho-diesterase	3.1.4.1	Phosphodiesterase 1, Type IV	<i>Crotalus atrox</i> (Western Diamond-back Rattlesnake) venom	ns	0.03 units mg ⁻¹ solid (one unit will hydrolyze 1 µmol bis- <i>p</i> -nitrophenyl phosphate per min at pH 8.8 at 37°C)	ns
Pant and Warman (2000)	Phospholipase C (phosphatidylcholine choline phosphohydrolase)	3.1.4.3	Type I	<i>Clostridium perfringens</i> (<i>C. welchii</i>)	One unit will liberate 1 µmol of water soluble organic P from egg yolk L-α-Phosphatidylcholine per min at pH 7.3 at 37°C	10–50 units/mg protein	ns
Pant and Warman (2000)	Nuclease (micrococcal)	3.1.31.1	ns	<i>Staphylococcus aureus</i>	ns	104 units/mg solid	ns
Pant <i>et al.</i> (2002)	ATPase	ns	ns	Rabbit kidney (<i>Sylvilagus</i> spp.)	ns	ns	ns

^ans, not specified or not available.

Controls (without addition of enzyme mixtures) are performed for each sample and the difference in P concentration between the incubated and unincubated samples is labeled as P released by the specific phosphatase. For example, alkaline phosphatase hydrolyzes labile monoester P (e.g., sugar phosphate, mononucleotides), phosphodiesterase hydrolyzes diester P (e.g., nucleic acids, phospholipids), and phytase hydrolyzes phytic acid.

The use of commercially available phosphatase enzymes to determine bioavailable organic P in marine waters and sediments was first reported by Strickland and Solarenzo (1966). Enzymatic techniques have confirmed the importance of organic P compounds in meeting the P needs of aquatic biota in inorganic P limited ecosystems. A number of studies have suggested that mineralization of organic P compounds in freshwater regimes is carried out by phosphatases released by both pro- and eukaryotic organisms (Flynn *et al.*, 1986). Similarly, the production of natural phosphatases either by roots or associated microorganisms in soil is a well-known process in the acquisition of P by plants. These enzymes catalyze the release of orthophosphate from a wide range of organic P compounds (Spier and Ross, 1978; Tadano *et al.*, 1993; Tarafdar and Claassen, 1988). Consequently, this method has been used to investigate the cycling of organic P in aquatic and terrestrial environments (Cooper *et al.*, 1991; Francko and Heath, 1979; Hino, 1989; Toor *et al.*, 2003). These studies have contributed to an improved understanding of the nature of these organic P species present in the soil and water and led to interest in the suitability of this technique to characterize P forms in organic wastes. He and Honeycut (2001) used different phosphatases to understand organic forms of P in pig and cattle manures. They first fractionated P in manures using Hedley sequential chemical fractionation method and then added different phosphatase enzyme mixtures in the sequential extracts to liberate the different forms of organic P. He and Honeycut (2001) observed that 39 and 17% of total P in H₂O extracts of pig and cattle manure, respectively, was phytic acid, whereas the NaOH extracts contained simple phosphomonoesters in the pig (43%) and cattle manure (15%). The nucleotide-like phosphodiesters (2–12%), and nucleotide pyrophosphate (0–4%) in NaOH extracts were present in smaller amounts. In their subsequent studies, He *et al.* (2003a,b; 2004a,b) improved the enzymatic hydrolysis method, by testing a large number of dairy, poultry, and swine manures.

Enzymatic hydrolysis has the potential to characterize different organic P species in wastes, however, a range of enzymes under a similar set of conditions need to be evaluated to establish a generalized procedure. This is necessary because researchers currently use different incubation times, incubation temperatures, and buffers, which makes comparison of results among different studies much more difficult.

F. NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

Due to advances in instrumentation in recent decades, NMR spectroscopy has been used for structure elucidation in organic and inorganic chemistry for complex biomolecules (such as proteins, enzymes, and nucleic acids) and has found its way into environmental chemistry and geochemistry (Nanny *et al.*, 1997; Wilson, 1987). Many of the NMR-active nuclei have been studied by environmental and soil researchers, particularly ^1H and ^{13}C (Chefetz *et al.*, 2002; Mao *et al.*, 2000, 2001; Preston, 1996), also ^{31}P (Amelung *et al.*, 2001; Cade-Menun, 2002, 2004; Cade-Menun and Preston, 1996; Condrón *et al.*, 1985; Guggenberger *et al.*, 1996; Hinedi *et al.*, 1989a; Leinweber *et al.*, 1997; Lookman *et al.*, 1996; Mahieu *et al.*, 2000b; Makarov *et al.*, 2002; McDowell *et al.*, 2002) and ^{15}N (Achtnich *et al.*, 1999; Mahieu *et al.*, 2000a; Zang *et al.*, 2001). The ^{31}P nucleus is ideally suited for NMR spectroscopy due to its high gyromagnetic ratio and high natural abundance (Condrón *et al.*, 1997) and has been used to characterize P in soils (Adams and Byrne, 1989; Condrón *et al.*, 1997; Magid *et al.*, 1996; Makarov *et al.*, 2002; McDowell *et al.*, 2002; Moller *et al.*, 2000; Sanyal and De Datta, 1992). NMR spectroscopy has been widely used to characterize organic P in manures (Hunger *et al.*, 2004; Leinweber *et al.*, 1997; Maguire *et al.*, 2004; Toor *et al.*, 2005a,b), composts (Frossard *et al.*, 2002), and biosolids (Hinedi *et al.*, 1989a,b). Nuclear magnetic resonance spectra can be recorded either in the solution or solid state. Drawbacks and advantages of both methods are discussed briefly in the following before outlining the main work pertaining to the investigation of P species in organic wastes.

1. Solution State Nuclear Magnetic Resonance Spectroscopy

The main advantage of ^{31}P solution or liquid state NMR over solid-state NMR is the significantly increased resolution of the spectra and thus the improved identification and quantification of P species in the sample. This is made possible by the rapid spinning of the sample and the resulting tumbling motion of the molecules in solution. This motion averages chemical shift anisotropies and field inhomogeneities, thereby narrowing the resonance signal (Drago, 1992). The extraction of organic wastes with alkaline extractants (e.g., NaOH-EDTA) offers the advantage of concentrating the P compounds in the waste and removing paramagnetic metal cations (such as Fe and Mn), which are associated with P and are known to greatly impair NMR analysis (Cade-Menun and Preston, 1996; Cade-Menun *et al.*, 2002; Smernik and Oades, 2002; Taranto *et al.*, 2000). Extraction sequences used

for the extraction of P species from soils have been extensively reviewed and compared by Cade-Menun and Preston (1996). The main problem in extracting environmental samples for NMR spectroscopy is the exclusive analysis of soluble P species, particularly orthophosphate and organic phosphate esters. By definition, this neglects the solid P minerals (e.g., Ca, Fe, and Al phosphates) that control solubility of P in environmental media. It is therefore used to most advantage for analysis of the distribution and transformation of organic P species in wastes. During extraction of wastes with alkaline extractants, there is the possibility of alteration of the P species by either hydrolysis of organic phosphate esters (Cade-Menun and Preston, 1996; Cade-Menun *et al.*, 2002; Turner *et al.*, 2003) or desorption of phosphate that is complexed on surfaces. Extraction therefore overestimates the fraction of orthophosphate and underestimates organic phosphate esters (Leinweber *et al.*, 1997).

Only a few researchers have used chemical extraction to analyze the P species in animal manure by ^{31}P -NMR. Crouse and coworkers (2000) used an alkaline extractant (0.25 M NaOH) combined with chelation of polyvalent metal ions and studied the influence of sample pH and temperature on the spectral resolution. They found that the spectral resolution was greatest at pH 10 and 20°C but did not attempt a peak assignment. Their research established important relations between chemical shift and line shape and pH and temperature.

Leinweber and coworkers (1997) employed two different extractants (0.5 M and 0.1 M NaOH) and compared the fractions of organic and inorganic P species observed in pig manure, chicken manure, and soils. They distinguished between orthophosphate, phosphate-monoesters, -diesters, teichoic acid (a glycosylated phospholipid common in bacterial cellwalls), and pyrophosphate. They observed a reduced fraction of organic phosphate diesters compared to monoesters in the 0.5 M NaOH extracts of both pig and chicken manure because of the alkaline hydrolysis of diesters to monoesters and possibly reduced extractability of diesters in the 0.1 M NaOH extracts (Table XIII). This illustrates the risk of creating artifacts that confound the use of NMR when using strong alkaline reagents to extract P species from organic wastes.

In their seminal publication, Hinedi and coworkers (1989a) characterized P species extracted from municipal biosolids and dairy manure using an array of aqueous and nonaqueous extractants. Of the reagents used, 0.5 M NaOH and a trichloroacetic acid/KOH extraction sequence appeared to be the most effective at extracting the hydrophilic species, while chloroform was best suited to extract lipophilic species. They assigned the signals to orthophosphate and various organic esters by comparison with literature values of chemical shifts and tentatively identified phospholipids including

Table XIII
Percent of Total P Concentrations Extracted with Different Chemical Extracts and with ^{31}P -NMR Spectroscopy in Animal Manures^a

	Liquid pig manure		Chicken manure	
	0.5 M NaOH	0.1 M NaOH	0.5 M NaOH	0.1 M NaOH
Orthophosphate	77	55	20	24
Monoester-P	13	12	65	40
Diester-P	9	33	14	35

^aAdapted from Leinweber *et al.* (1997).

phosphatidylcholine, phosphatidylethanolamine, and phosphatidylserine. Their results indicated that the distribution pattern of P species depends on the biosolids digestion process, with aerobic digestion resulting in a higher fraction of organic phosphate esters than anaerobic digestion, which exclusively contained orthophosphate.

The characterization of P species in animal manures has been advanced by Turner (2004) and Turner *et al.* (2003), who optimized the extraction protocol and assembled a vast data base of NMR spectra of diverse organic and inorganic P compounds for the identification of P species in alkaline extracts of soils, animal wastes, and biosolids.

Case Study: Linking Phosphorus Forms in Dairy Diets with Feces and Manures Characterizing P in animal diets and manures is fundamental for the holistic management of animal feeding operations. Studies have clearly demonstrated that excess of P in diets results in P rich manures. Currently, there is a lack of information about the different forms of P present in a continuum from animal diets to manures. In a first comprehensive study to obtain information about the process of P utilization from diets to manure, Toor *et al.* (2005a) used solution state NMR to identify various inorganic and organic compounds of P in dairy diets, feces, and manures collected from Northeastern and Mid-Atlantic United States. They found that the concentrations of orthophosphate were more variable in diets (36–67%) than in feces (51–74%) and manures (63–77%) (Table XIV). This showed that the animal digestion process and subsequent on-farm practices resulted in more uniform manures in terms of proportion of P forms despite the variations in dietary orthophosphate. Toor *et al.* (2005a) also noted that phospholipids and DNA were lower in diets but greater in feces because of excretion of microbial cells from the digestive tract, which were easily decomposed in manure pits.

Table XIV
Functional Classes (*n* = 6) of Phosphorus Determined in NaOH–EDTA Extracts of Diets, Feces, and Manures by Solution ³¹P-NMR Spectroscopy^a
(in Percentage of Total Phosphorus Extracted with NaOH–EDTA)

	Inorganic P			Organic P					
	Orthophosphate	Pyrophosphate	Unidentified	Orthophosphate monoesters		Orthophosphate diesters			Phosphonates
				Phytic acid	Unidentified	Phospholipids	DNA	Unidentified	
Diets									
Mean	55 ± 11.6a	1.7 ± 0.7a	0.8 ± 0.01a	32 ± 8.5a	8.6 ± 3.4a	2.3 ± 0.7a	1.0 ± 0.4a	1.8 ± 0.00a	–a
Range	35–68	0.8–2.6	0–0.8	21–47	5.2–13.9	1.6–3.5	0.8–1.8	0–1.8	0
Feces									
Mean	62 ± 8.3ab	1.4 ± 0.4a	0.8 ± 0.1a	18 ± 6.2b	8.7 ± 4.4a	5.6 ± 1.5b	2.1 ± 0.4b	1.5 ± 1.0a	1.0 ± 0.3b
Range	51–74	0.8–1.8	0–0.8	11–29	4.4–17.9	3.6–7.5	0–2.6	0–3.2	0.8–1.6
Manures									
Mean	73 ± 4.5bc	1.9 ± 0.3a	0.8 ± 0.1a	9 ± 3.5c	9.0 ± 2.2a	3.7 ± 0.9c	1.9 ± 1.3b	1.4 ± 0.4a	1.0 ± 0.3b
Range	63–77	1.6–2.5	0–0.9	5–14	6.1–12.5	2.4–5.0	0–4.5	0–1.7	0–1.6
LSD (5%)	12	0.7	0.5	9	4.6	1.4	0.8	1.2	0.4

^aAdapted from Toor *et al.* (2005a).
Means followed by different letters in the same column are significantly different at *p* < 0.05.

2. Solid State Nuclear Magnetic Resonance Spectroscopy

Unlike solution NMR spectroscopy, solid-state ^{31}P -NMR spectroscopy analyzes the P species in the entire, undisturbed sample without extraction. Although air drying or oven drying at low temperature and grinding of the sample is necessary for uniform distribution and homogeneous spinning of the rotor in the magic angle spinning (MAS) probe (Condrón *et al.*, 1997), it can allow the retention of some water in the sample (Hunger *et al.*, 2004) thereby limiting the temperature induced changes of the P species. However, without extraction with alkaline extractants, an efficient removal of paramagnetic metal cations is not possible. Therefore, solid phosphate species as Fe phosphate and phosphate adsorbed to Fe and Mn mineral phases are invisible to the NMR spectrometer. Attempts have been made to extract paramagnetic metal cations from soil samples (Lookman *et al.*, 1996) and biosolids (Hinedi *et al.*, 1989a) using citrate–bicarbonate–dithionite or oxalate extractants but a concomitant extraction of Al and P species could not be excluded.

Due to the random orientation of nuclear momenta in solid samples, chemical shift anisotropy of the observed nucleus leads to a broad, unresolved solid-state NMR pattern. This can be overcome by spinning the sample about the magic angle (54.7°) relative to the static magnetic field at high spinning frequencies (5–10 kHz or 300,000–600,000 rpm) (Condrón *et al.*, 1997; Drago, 1992). The only remnants of the broad NMR pattern without MAS are spinning side bands (SSB), which are spaced in multiples of the spinning frequency on both sides of the resonance signal and which can also be used for diagnostic purposes. The occurrence and intensity of SSBs depends on the symmetry of the chemical environment of the observed nucleus and its perturbation by chemical bonds (see Bleam *et al.*, 1989). Single pulse MAS-NMR with and without proton decoupling, which further narrows the resonance signal and increases the resolution, has become the standard technique for solid samples.

Another technique that is useful for environmental samples containing several P species that differ in their degree of protonation, is cross-polarization under MAS conditions (CP-MAS) (Hartmann and Hahn, 1962; Pines *et al.*, 1973). A macroscopic magnetic momentum is created in the protons, which is transferred to the P nuclei during a contact time in the range of milliseconds. The efficiency of this transfer is dependent among others on the distance between the proton and the P nucleus. Protonated P-species or phosphate groups in solid phases that have protons or water molecules on fixed crystallographic positions in close proximity are therefore selectively enhanced, while unprotonated P-species are suppressed. The combination of CP-MAS and single pulse MAS-NMR often allows for an unambiguous identification of P species.

Hinedi and coworkers (1988, 1989b) investigated P species in wastewater biosolids and biosolids-amended soils using MAS-NMR. They identified Ca phosphate phases and pyrophosphate (–9 ppm) in the biosolids-amended soils and the biosolids itself, and also Al phosphate in the biosolids. The Ca phosphate phase (3.0 ppm) in biosolids-amended soil was tentatively identified as a carbonated, poorly ordered apatite. Intense SSBs were observed that could be reduced by using a citrate–bicarbonate–dithionite extractant and were therefore attributed to perturbation of the tetrahedral symmetry of the phosphate group by paramagnetic metal cations in the samples.

Duffy and van Loon (1995) investigated the P speciation in biosolids using solid state NMR spectroscopy by comparing the chemical shift with that of Al hydroxyphosphate precipitates aged for various times and dried at different temperatures. They found that phosphate species associated with Al generally have ^{31}P chemical shifts in the range from –7 to –30 ppm, with the less condensed Al phosphate polymers appearing at the higher end of this range. Their results correlated Al and P species in the treated product with the biosolids treatment.

Frossard and coworkers (1994) studied P speciation in urban biosolids by sequential extraction (H_2O , NaHCO_3 , NaOH , HCl) with subsequent NMR spectroscopic analysis of the solid residue after each extraction step. They found a complex mixture of Ca phosphate phases, mainly Ca octaphosphate and apatite (2–3 ppm) that were only dissolved during the acid extraction step, and a poorly ordered Al phosphate (–13 ppm), tentatively identified as wavellite, which remained in the residue after the extraction sequence. Of the latter they were however not sure, whether or not it was an artifact, created by reprecipitation during the extraction steps. Also, the presence of Fe phosphate phases could not be excluded, as observed earlier by Hinedi *et al.* (1989b). In a study of composted solid organic wastes, Frossard *et al.* (2002) identified Ca phosphate phases but were unable to resolve the complex signal of organic phosphate species. These species were also observed by Hunger *et al.* (2004) in a study of unamended and alum-amended poultry litter by a combination of single pulse MAS and CP-MAS-NMR spectroscopy.

Case Study: Phosphorus Speciation in Unamended and Alum-Amended Poultry Litters Samples of poultry litter were obtained from an extensive on-farm evaluation of the effectiveness of alum as an amendment for poultry litter (Sims and Luka-McCafferty, 2002). Poultry feed contained dicalcium phosphate and Ca carbonate. The poultry litter consists of the feces and the bedding material, mainly wood chips and sawdust, and had neutral to slightly basic pH values. In the alum-amended litter, the pH initially dropped to pH 5–5.5 after addition of alum and slowly increased over time

as more feces were added, reaching slightly lower values than the unamended litter. This behavior was attributed to the hydrolysis of Al^{3+} , which leads to the formation of an amorphous Al hydroxide. Due to the storage under warm, humid conditions in the chicken houses, the organic material was partially humified. Considering the elemental composition and the pH of poultry litter (Jackson *et al.*, 2003; Sims and Luka-McCafferty, 2002), litter P chemistry was expected to be dominated by interactions with Al (only in the amended samples), Mg, and Ca, and by organic phosphate compounds derived from metabolic processes. Minor components in litter that form stable compounds with P are Fe, Mn, and Zn.

The single pulse MAS-NMR spectra of the unamended and the alum-amended poultry litter samples are presented in Figs. 6 and 7, respectively, the CP-MAS-NMR spectra in Figs. 8 and 9, respectively. The spectra show the same complexity as those found in the literature, but unlike the previously published solid-state spectra, they contain sharp signals that resemble the peaks found in solution NMR spectra. The sharp peak with a chemical

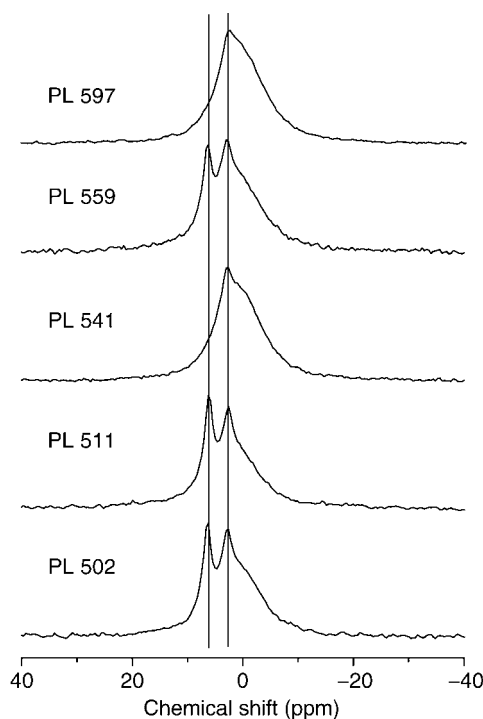


Figure 6 Single-pulse, proton decoupled ^{31}P -MAS-NMR spectra of unamended poultry litter samples; chemical shifts of the marked peaks are: $\delta = 6.4$ and 2.8 ppm.

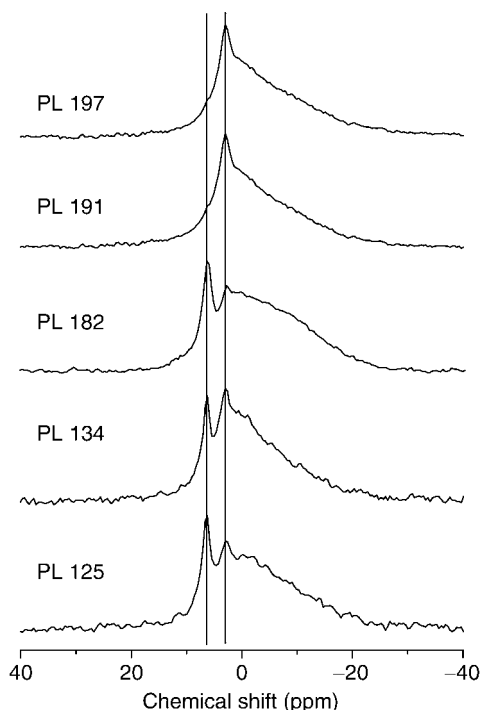


Figure 7 Single-pulse, proton decoupled ^{31}P -MAS-NMR spectra of alum-amended poultry litter samples; chemical shifts of the marked peaks are: $\delta = 6.4$ and 2.8 ppm.

shift at $\delta = 6.4$ ppm is also selectively enhanced by cross-polarization, which indicates a phosphate group that is either protonated or bound by hydrogen bonds to surrounding water molecules. Considering the chemical shift, which has been attributed to orthophosphate in alkaline extracts of soils and biosolids, it was incorrectly concluded by Hunger *et al.* (2004) that the corresponding P species was singly protonated orthophosphate (HPO_4^{2-}) forming hydrogen bonds to water molecules in the organic matrix or to organic functional groups. Powder x-ray diffraction (XRD) however revealed the presence of the ammonium magnesium phosphate mineral struvite ($\text{MgNH}_4\text{PO}_4 \cdot 6 \text{H}_2\text{O}$) (Hunger, unpublished data), which has one characteristic sharp line at 6.35 ppm in the NMR spectrum (Bak *et al.*, 2000). To our knowledge, this compound has not been identified in animal manure before and is likely to represent an important P pool in poultry litter (compare Figs. 8 and 9).

The signal at $\delta = 2.8$ – 3.0 ppm is also relatively sharp. Peaks in this chemical shift region in solid state NMR spectra of organic wastes have

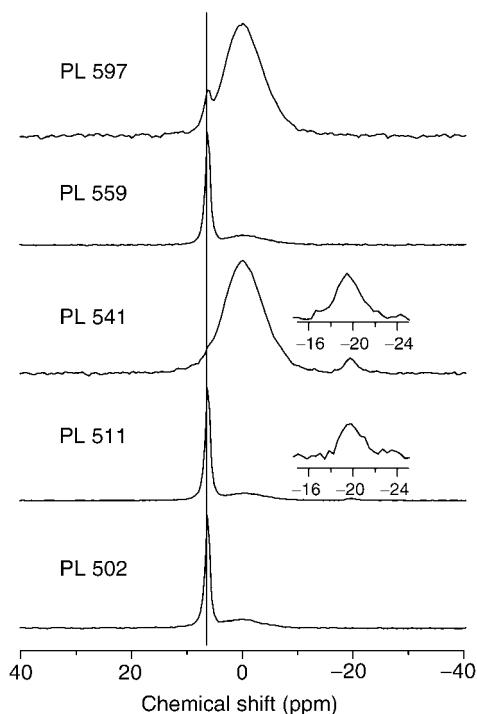


Figure 8 CP-MAS ^{31}P -NMR spectra of unamended poultry litter samples; the insets show enlargements of the chemical shift region indicated; the chemical shift of the marked peak is $\delta = 6.4$ ppm.

been attributed to condensed Ca phosphate phases, such as hydroxylapatite, carbonated apatite, and octacalcium phosphate (Frossard *et al.*, 1994, 2002). A signal in the same chemical shift region was observed by Hinedi *et al.* (1992) in the NMR spectra of phosphate sorbed to CaCO_3 . Calcium carbonate may precipitate in poultry litter during storage in the chicken houses and might also pass unchanged through the birds' digestive tract. It can therefore not be unambiguously decided whether the Ca phosphate phase observed is a poorly ordered apatitic or octacalcium phosphate phase or a Ca phosphate surface precipitate on CaCO_3 .

Similar to the results of Frossard and coworkers (1994, 2002), the organic phosphate species are unresolved and show one broad signal centered between +1 and -1 ppm. This is easily explained by the wide variety of chemical environments of the phosphate group in organic phosphate species (phospholipids, nucleic acids, metabolites, etc.) and their limited mobility. Rapid tumbling of the molecules in solution NMR (not available in solid state NMR) averages the different molecular conformations and leads to

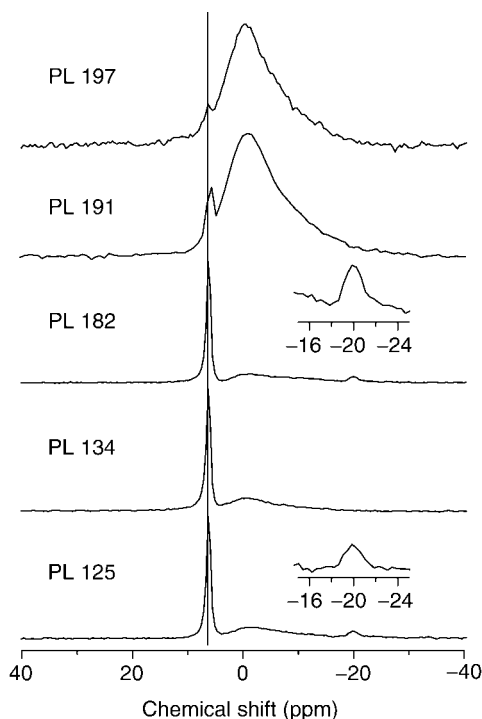


Figure 9 CP-MAS ^{31}P -NMR spectra of alum-amended poultry litter samples; the insets show enlargements of the chemical shift region indicated; the chemical shift of the marked peak is $\delta = 6.4$ ppm.

sharpened signals. Contributions in this chemical shift region may also come from orthophosphate complexed with a variety of metal cations. Turner and coworkers (1986) found a linear correlation between chemical shift and the electronegativity of the cations in several metal phosphates, with the less electronegative metals shifting the phosphate peaks to more positive values. Because of the inhomogeneity of the material, a unique chemical environment of the P nucleus is not given, which results in a broad resonance peak.

The very weak signal at $\delta = -20$ ppm that can be found in some CP-MAS spectra of both amended and unamended poultry litter samples, is an artifact that can be traced to a contamination of the rotor in the NMR with variscite ($\text{AlPO}_4 \cdot 2\text{H}_2\text{O}$).

The alum-amended samples exhibited a broad signal in the chemical shift range of -4 to -10 ppm, which appeared as a shoulder on the negative side of the signal of the organic and inorganic phosphate species. The fact that this signal is exclusively present in the spectra of the alum-amended samples

indicated that it could be attributed to a P species created by either Al or its hydrolysis products. This could be corroborated by ^{31}P TRAPDOR (transfer of polarization in double resonance), an NMR pulse sequence that selectively detects pairs of Al and P nuclei (Lang *et al.*, 2001; Rong *et al.*, 1998; Schaller *et al.*, 1999; van Eck *et al.*, 1995).

The presence of a condensed, crystalline Al phosphate phase can be excluded due to the absence of a signal in the corresponding chemical shift region of -19 to -24 ppm (Bleam *et al.*, 1989; Duffy and van Loon, 1995). Less condensed Al phosphate phases, such as wavellite (Araki and Zoltai, 1968), have been reported to have chemical shifts of -11 to -13 ppm (Bleam *et al.*, 1989) and have been suggested as the phases present in soils (Frossard *et al.*, 1994; McDowell *et al.*, 2002). The signals in the spectra of alum-amended poultry litter are found in the range from -4 to -10 ppm, but a tailing to -20 ppm can also be observed (Fig. 7). Both surface complexes and amorphous, uncondensed Al phosphate minerals, such as poorly ordered wavellite, are therefore suggested by NMR analyses to be present in the alum-amended poultry litter.

In the single pulse MAS-NMR spectra of the alum-amended poultry litter samples (Fig. 7), the peak attributed to phosphate adsorbed to $\text{Al}(\text{OH})_3$ (-4 to -10 ppm) contributes upwards of 50% to the overall peak intensities. This clearly shows that 50% and more of the total phosphate in the samples are bound to $\text{Al}(\text{OH})_3$ and therefore scarcely water extractable. Only a minor proportion is present as weakly bound phosphate and is expected to be readily water soluble.

The spectra of the unamended poultry litter samples (Fig. 6) are dominated by the unresolved signal of various disordered organic and inorganic phosphate species (60–80%), while Ca phosphate and struvite comprise only a small part. This indicates that most of the phosphate bound to $\text{Al}(\text{OH})_3$ in the alum-amended samples originates from the phosphate species, which give rise to the unresolved signal at approximately 0 ppm, and Ca phosphate. The hydrolysis of organic phosphate and the dissolution of Ca phosphate are therefore important processes at the acidic pH values initially reached upon application of alum.

These results could be confirmed and expanded in a follow-up study combining sequential chemical extraction and solid-state ^{31}P -NMR spectroscopic analysis of the solid residues in between extraction steps. Using a single sample from the same group of poultry litter samples described by Sims and Luka-McCafferty (2002), Hunger and coworkers (2005) were able to demonstrate changes in P speciation that occur during chemical extraction, mainly sorption reactions to $\text{Al}(\text{OH})_3$. Comparing the amounts of Ca phosphate determined by solid state NMR and by sequential chemical extraction, they also found that sequential chemical extraction overestimated the fraction of Ca phosphate phases by an order of magnitude. Although

this result is only supported by the one sample investigated, serious doubt was cast on the accuracy of quantification of P species in poultry litter by sequential chemical extraction.

Comparison of Solid State and Solution State Nuclear Magnetic Resonance Spectroscopy As has been illustrated in the preceding paragraphs, both solution and solid-state ^{31}P -NMR spectroscopy have their advantages and disadvantages when used to investigate P species in organic wastes. Solution state NMR of alkaline extracts is ideally suited for the investigation of organic P species in the wastes and their transformations in soils with different waste treatments. However, care must be taken to minimize hydrolysis of organic P species. The largest disadvantage of solution NMR spectroscopy is that different inorganic P species in the organic wastes cannot be separated due to the required extraction of samples with alkaline extractants. Similarly, phosphate surface complexes on Al and Fe (oxy)hydroxides are only desorbed under alkaline conditions of extended reaction times (Hedley *et al.*, 1982a). While solution state NMR can be of great benefit to characterize organic P species, solid state NMR is more suited to study the inorganic P species in the wastes. Using mild drying conditions, it is possible to maintain humidity levels in the waste that allow for almost *in situ* sample conditions during solid-state NMR spectroscopy. Both solution- and solid-state NMR methods are quantitative and can be used to characterize P species or track changes in P speciation, such as microbial transformations, hydrolysis, or dissolution in the wastes.

G. X-RAY ABSORPTION NEAR EDGE STRUCTURE SPECTROSCOPY

X-ray absorption spectroscopy (XAS) is an analytical tool that utilizes synchrotron radiation to determine atomic scale chemical information in samples and is widely used to study the speciation of trace metals in soils and soil components. A detailed explanation of XAS theory and instrumental techniques can be found in Fendorf and Sparks (1996). In an XAS experiment, x-rays are absorbed by an atom at certain energies, causing a core electron to be ejected from the central atom. This ejected electron is called a photoelectron. As the electron is ejected, a higher-energy electron in the atom will fill the core vacancy. Since this electron has higher energy than the core position it is filling, it releases the excess energy in the form of fluorescence. The energy with which fluorescence occurs is characteristic for each electron of an atom, so XAS is element specific. Most XAS studies are referred to as taking place at a particular "edge," which is another way of referring to the energy used. For example, the P K-edge (the energy needed to expel a P 1s electron) is ~ 2150 eV (depending upon oxidation state). An

XAS experiment typically measures the x-ray absorbance of a sample over a certain energy range to generate an absorption spectrum. This absorption spectrum can be divided into two regions: the near edge and the extended region.

The XANES spectroscopy is the region before the absorption edge to around 100 eV past the edge. The XANES spectra are typically very sensitive to the oxidation state of the central atom and to changes in coordination number from tetrahedral to octahedral. Past the edge, the XANES region is typically dominated by resonance and multiple scattering contributions. The XANES spectra of unknown and environmental samples are typically analyzed by comparison to standards, a technique known as “fingerprinting.” However, newly developed computer packages can now model XANES data by comparison with theoretical standards. In contrast, the extended portion of the spectrum is dominated by oscillations due to longer-range interactions between photoelectrons and other atoms. The XAS studies, which utilize these oscillations to determine chemical information about the sample, use a technique known as extended x-ray absorption fine structure (EXAFS) spectroscopy. The EXAFS data are analyzed by fitting it to theoretical scattering paths and the spectra provide information on coordination number, the identity of nearest neighbors, and bond distances.

X-ray absorption spectroscopy provides a molecular scale method to probe the local chemical environment of P in heterogeneous samples such as soils and organic wastes. EXAFS spectroscopic studies at the P K-edge are possible (Rose *et al.*, 1997) but are currently of limited use in soils because P concentrations in soil samples are often too low for EXAFS and many of the common cations associated with phosphate in soil (Al, C, Ca, and Si) are extremely weak backscatterers. P K-edge EXAFS studies will likely become more common as beamline configurations and detectors are upgraded and as the intensity of synchrotron light sources increases.

In contrast, XANES spectroscopy provides a method to probe the speciation of P in animal manures and biosolids with minimal sample modification and much simpler data reduction. Unlike many other routine spectroscopic tools, XANES can be employed with heterogeneous samples (e.g., soils and manures) and one can analyze samples *in situ* (with normal amounts of water at room temperature and pressure). Fingerprinting techniques work well with P K-edge XANES due to the fact that there are three different features of phosphate XANES spectra that are affected by local chemical environment: (i) intensity and position of a pre-edge peak, (ii) position of the white line energy, and (iii) resonance features past the edge.

The effects of local chemical environment on phosphate XANES spectra, pre-edge intensity and white line position, have been studied using a variety of mono-, di-, and tri-cationic metal phosphates. Franke and Hormes (1995) studied the effects that metal substitution has on P K-edge XANES spectra,

and found that changing the identity of the metal cation bound to the phosphate tetrahedron affected the P XANES spectra in a systematic manner. The intensity and position of a pre-edge peak changed as metals were varied, and the position of the white line peak was also affected. Past the edge, continuum resonances in the spectra were attributed to a combination of multiple scattering effects in the first coordination shell and to the interactions with atoms at higher coordination shells. In an especially insightful study, Okude and coworkers (1999) used P K-edge XANES to study the first row transition series of metal-phosphates and then correlated spectral features with the 3d electron structure of the metal phosphates (Fig. 10). It was determined that the intensity and position of a pre-edge peak and the position of the main peak could both be correlated with the number of 3d electrons in the transition metal. The position of the white line (main absorption peak) increases linearly with increasing number of d electrons in the metal center. The position of the white line peak, therefore, is a clue to the bonding environment of phosphate in XANES samples. Another spectral feature that changes systematically with metal electronic configuration is the pre-edge peak intensity and position. This pre-edge peak is attributed to hybridization/orbital mixing between 3p orbitals of phosphate and 3d and 4s orbitals of the transition metals. As the number of d electrons is increased, the intensity of this peak decreases because fewer d orbitals are available for mixing with P orbitals.

The effect of mineral structure upon resonance features in P XANES spectroscopy has not been systematically evaluated, but various researchers have provided reference spectra of many different CaPO_4 , AlPO_4 , and FePO_4 phases. One complication is that some researchers have not collected data very far past the absorption edge and so it may be difficult to use their results as standards. The XANES spectra of many common phosphate minerals and adsorbed phosphate species are collected in Fig. 11. The important features to note in the Ca phosphate minerals are that the XANES spectra change considerably as structure of the Ca phosphate phase changes. For monetite (a dicalcium phosphate phase), there is a single peak ~ 4 eV past the white line and an otherwise relatively featureless spectrum. For the other Ca phosphates, a shoulder is present at 2 eV past the edge that increases in intensity as the Ca phosphate phases become more crystalline and less soluble. This shoulder is not observed in other phosphate minerals and can be used to identify more crystalline Ca phosphates reliably in mixed samples. There is an additional peak that appears between 6 and 8 eV past the edge due to resonance features in Ca phosphate minerals for all phases other than monetite, and some shifts in the shape of the large oxygen oscillation, but these XANES features are much less diagnostic than the shoulder in apatite. For Al phosphate minerals (variscite, wavelite, and amorphous aluminum phosphate) there is a peak

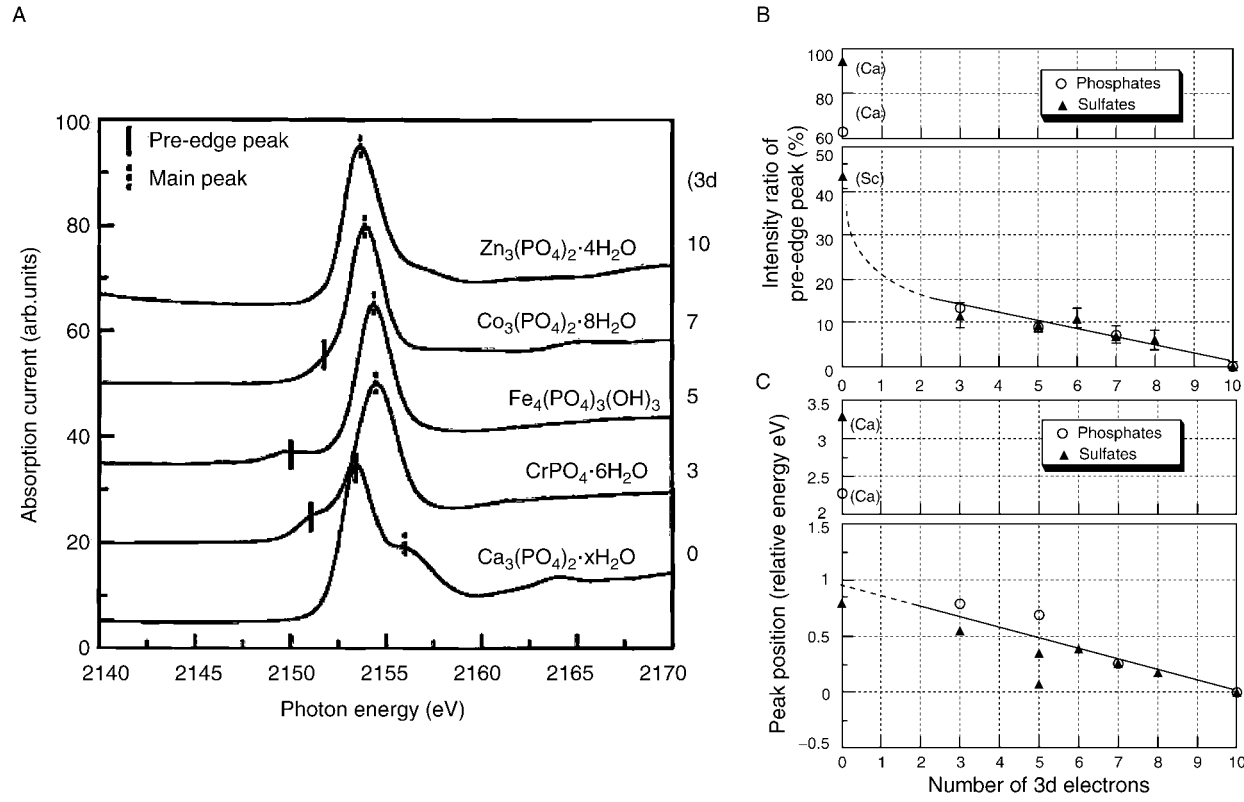


Figure 10 Effect of 3d metal electron configuration on phosphate pre-edge peak intensity and white line peak position. From Okude *et al.* (1999).

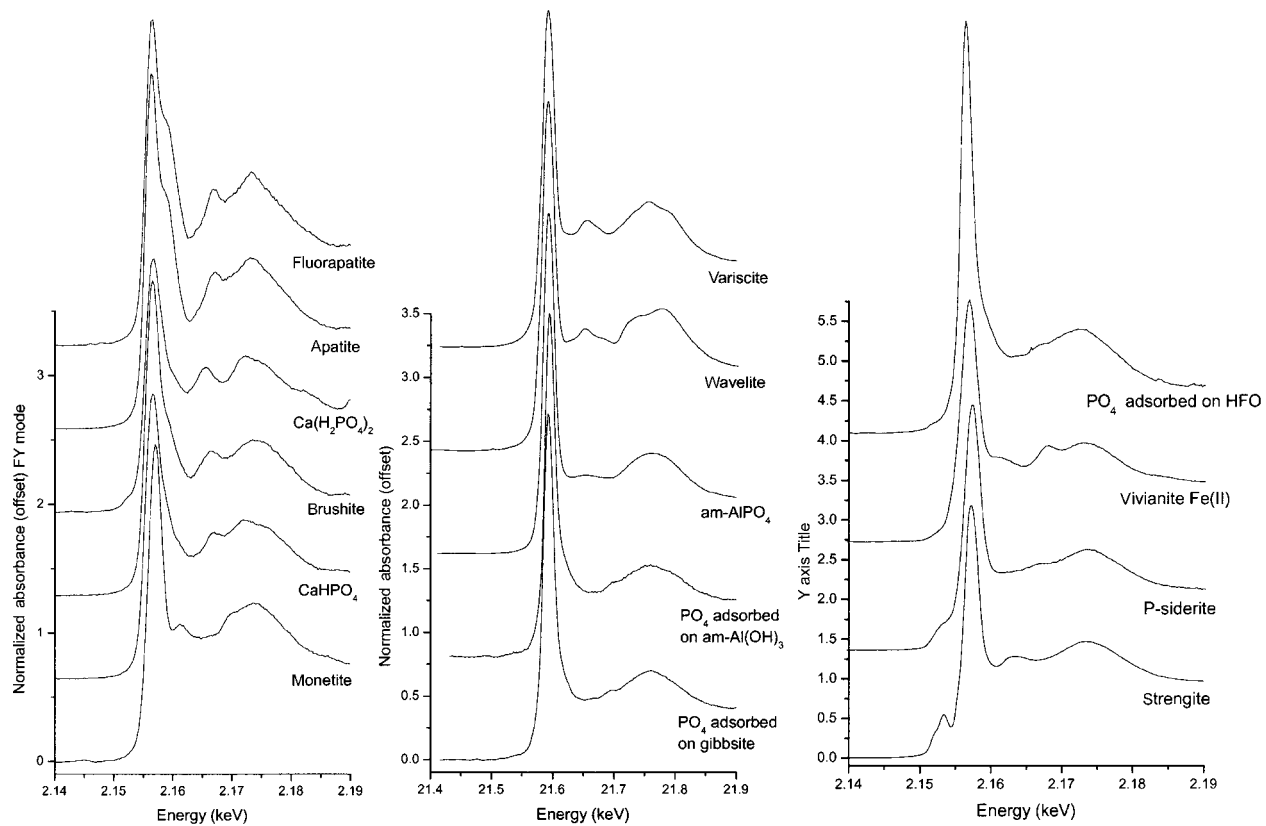


Figure 11 XANES spectra of reference CaPO₄, AlPO₄, and FePO₄ materials.

at approximately 5 eV past the white line energy that is present in all minerals. This peak is absent in the adsorption samples, and therefore can be used to distinguish adsorbed from precipitated phosphate. This feature is also different enough from the Ca phosphate features to distinguish Al phosphates from Ca phosphates in mixed systems. For Fe phosphate materials, the oxidation state of Fe has a large role on the XANES spectral features. The Fe(III) phosphate minerals have a strong pre-edge feature due to orbital mixing that can be seen very clearly in strengite, P-siderite, and weakly in the adsorption sample. This feature is absent in the Fe(II) phosphate mineral vivianite.

Several researchers have successfully utilized these diagnostic XANES features of phosphate minerals to study phosphate chemistry in soils and manures. Hesterberg *et al.* (1999) used XANES spectroscopy to study adsorbed and mineral forms of phosphate. They found XANES spectroscopy could distinguish phosphate adsorbed on Fe and Al oxides from Fe and Al phosphate precipitates. They also collected XANES spectra for a variety of Ca phosphate minerals and concluded that phosphate in a soil sample from North Carolina was in the form of dicalcium phosphate. Peak *et al.* (2002) used P, S, and Ca K-edge XANES spectroscopy to perform speciation of unamended and alum-amended poultry litter samples, as discussed in the following Case Study 1. Both of these studies were qualitative in nature and merely compared the spectral signature of standards to different samples. More advanced P XANES spectroscopic analysis techniques have (linear combination fitting or LC XANES) been used to quantitatively assess P speciation in soils (Beauchemin *et al.*, 2003) and in poultry litters and turkey manures (Toor *et al.*, 2005c). Both of these studies assessed the composition of natural samples by fitting their spectra to a group of reference standards. The application of LC XANES to determine the relationship between animal diet and P speciation in manures will be discussed in Case Study 2. This form of speciation is complicated by the fact that fluorescence spectra of concentrated samples are distorted by self-absorption effects. This is a well-known issue with XAS experiments in the 2000–4000 eV range, and researchers using P K-edge XANES should be well versed in the possible pitfalls associated with these problems.

Self-absorption occurs in an XAS sample when concentrations of the element of study are high enough that the ejected core electron interacts with other atoms in the sample matrix rather than being released into the continuum. This has the effect of distorting the XANES spectrum around the white line energy and decreasing the main peak's intensity (similar to saturation). There are several strategies to deal with self-absorption effects. In hard x-ray experiments, the easiest method of dealing with self-absorption is to use thin film samples and collect data in transmission mode ($\log I/I_0$) with a detector placed directly before (I_0) and after (I) the sample. This

sampling environment requires that some of the x-ray beam must pass completely through the sample. At the P K-edge, the depth of penetration is only a few microns, and transmission mode data collection is not possible. There are, however, two experimental approaches that one can take to eliminating self-absorption issues in their XANES data. First of all, one can dilute all samples to the same P concentration (in mg kg^{-1}) with a nonabsorbing powder, such as BN_3 , and then collect data in fluorescence mode. Another possibility is to conduct the analysis under ultra high vacuum (UHV) conditions and collect a total electron yield (TEY) spectrum of the surface only. The sampling depth of TEY experiments will depend on the energy range, but researchers previously determined that at the Si K-edge (1840 eV) TEY probes 60 nm deep versus several hundred nm for the fluorescence yield (FY) measurements (Kasrai *et al.* 1996). As a result, TEY spectra are typically free of self-absorption effects. Figure 12 shows a comparison of phytic acid reference standard analyzed as an aqueous solution in fluorescence mode, and as a powder with TEY and FY measurements. The decreased absorbance at the white line of the fluorescence samples is caused by self-absorption. The aqueous sample is considerably less distorted due to a lower P concentration in this sample compared to the salt. If the aqueous and salt samples were further diluted, their spectra would approach the intensity of the TEY spectrum. If one were to use the phytic acid salt sample collected in fluorescence mode as a standard for linear combination XANES analysis, the results would be much different from using the TEY or aqueous standard. The aqueous phosphate spectrum is included in Fig. 12 to illustrate another point. There are no clear spectral features of phytic acid that distinguish them from aqueous inorganic phosphate. Subtle differences in the intensity of the white line peak and the shape of the oxygen oscillation are present in phytic acid, but there is nothing that makes it possible to conclusively separate phytic acid from aqueous inorganic phosphate. This makes P K-edge XANES spectroscopy poorly suited to conclusive identification of organic phosphates.

From the above discussion, one might infer that TEY spectra are always the preferred choice of analysis for powder samples. There is however another complication that should be addressed. Since TEY only probes the top ~ 60 nm of the sample, it is possible for the surface structure of the samples to differ from the bulk structure of the material. Two examples of this are shown in Fig. 13. In the case of vivianite (a natural ferrous phosphate mineral), the surface was oxidized due to exposure to the atmosphere and the TEY spectrum resembles that of strengite (a ferric phosphate mineral) from Fig. 11. In the case of monetite (a naturally occurring dicalcium phosphate mineral), there is no change in oxidation state, but the TEY spectrum has much less structure than the FY spectrum. One explanation for this is that the mineral is relatively ionic in nature and that the surface

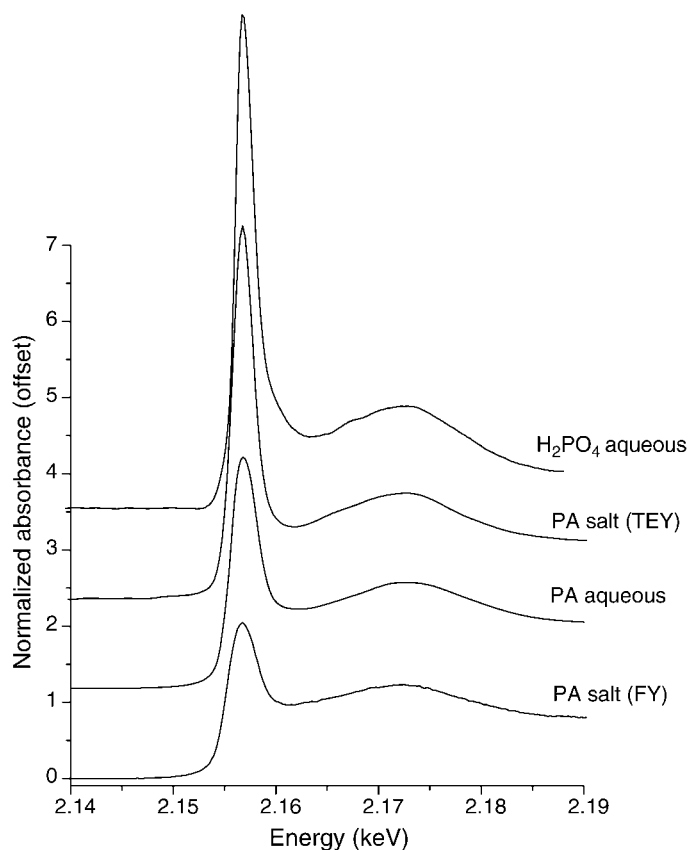


Figure 12 XANES spectra of phytic acid collected in fluorescence yield (FY) on an aqueous solution and a salt as well as a total electron yield spectrum of the phytic acid salt. The decreased intensity of the white line peak in the fluorescence samples is caused by self-absorption effects.

layer of phosphate, when no longer coordinatively saturated with calcium, is relatively weakly bound. This could also explain the high solubility of dicalcium phosphate compared to other calcium phosphate minerals.

The above discussion should highlight the fact that no sampling method is perfect and that care must be made in both sample preparation and data analysis. Which method of data collection is most useful depends upon the physical state of the samples (UHV beamlines require dried samples for TEY measurements) and the amount of beam time available (dilution to suitably low concentrations for fluorescence studies will demand much longer data collection times to achieve the same signal-to-noise ratio).

Detection limits are dependent on the type of detectors and sample chamber chosen, but can often make performing phosphate speciation in

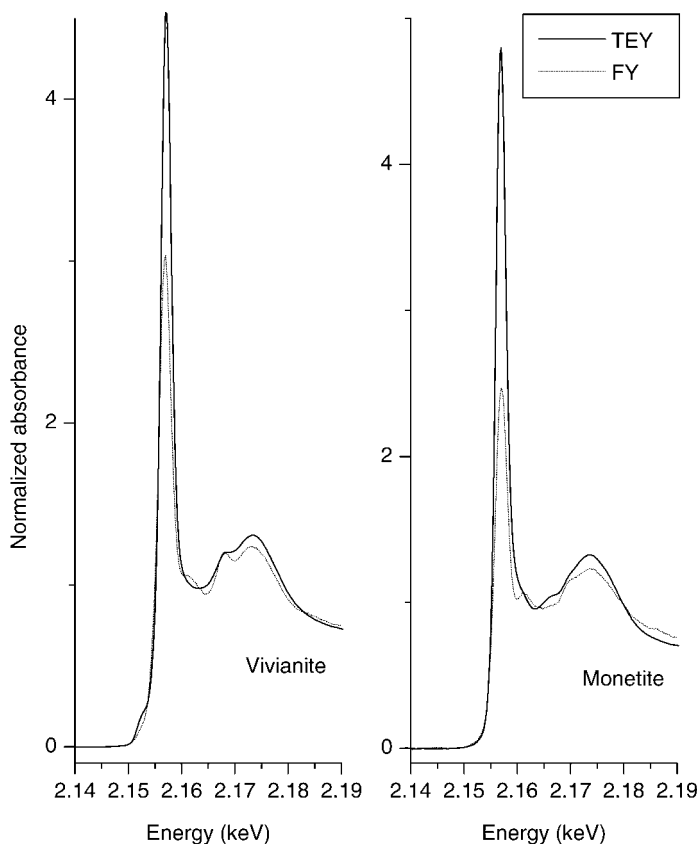


Figure 13 Examples of phosphate samples where total electron yield data and fluorescence yield (FY) data differ significantly due to surface modification. In the vivianite Fe(II)PO_4 sample, surface oxidation has occurred that results in a total electron yield spectrum consistent with Fe(III)PO_4 . In the monetite sample, the total electron yield spectrum appears substantially different from the bulk fluorescence in structure. This may be evidence that the surface layers of phosphate are very weakly bound in this mineral.

natural soils with XANES difficult. Conversely, self-absorption can significantly distort the XANES spectrum if concentrations are too high. Another current limitation is the availability of a suitable beam lines for environmental samples that operate in the 2–3 keV energy range. For chemical speciation in soils, UHV beamlines are not generally recommended because analysis of aqueous references, moist references, and samples is the primary goal. However, manure, biosolids, and soil samples are typically air-dried and sieved prior to chemical analysis and so the limitations of UHV beamlines may not be as large of an issue as with typical environmental XAS

samples. And as mentioned previously, TEY mode data collection has some advantages for quantitative XANES analysis.

Case Study 1: Natural and Alum-Amended Poultry Litter

Peak and coworkers (2002) have shown that P K-edge XANES can provide important information about the chemical speciation of P in animal manures. Beamline X-19A of the National Synchrotron Light Source at Brookhaven National Laboratory was utilized for all XANES experiments. The XANES spectra of aqueous and organic phosphates as well as Ca phosphates, Al phosphates, and phosphate adsorbed on Al oxides were all collected as references for chemical fingerprinting. The XANES spectra of many different P reference materials were previously shown in Fig. 11 (Ca, Al, and Fe phosphates) and Fig. 13 (phytic acid). The important spectral features to keep in mind from those standards are: (i) All Al phosphate precipitates have a slight peak in the 2163–2165 eV range, which can be used to distinguish phosphate adsorbed on Al oxides from Al phosphate precipitate phases, and (ii) all Ca phosphates have a spectral feature near the white line position that appears as a peak or a shoulder between 2160 and 2162 eV.

The XANES spectra of poultry litter samples were then compared to the reference compounds to determine the chemical forms of P. Two primary types of litter collected from Delaware poultry houses were analyzed: unamended litter and aluminum-sulfate amended litter (Sims and LukacMcCafterty, 2002). Spectra of a representative unamended and alum-amended poultry litter are shown in Fig. 14. This figure also includes spectra of potentially occurring P reference standards for comparison. In the unamended sample, the P XANES spectrum seems to contain a mixture of aqueous phosphate, organic phosphate, and dicalcium phosphate. However, in the alum-amended sample, dicalcium phosphate peaks are no longer present. Instead, the P XANES spectrum is consistent with a mixture of phosphate adsorbed on Al oxide, organic phosphate, and aqueous phosphate. No evidence of Al phosphate precipitation could be seen in any alum-amended litter. There are several reasons that Al phosphate precipitation may not occur in alum-amended litter. Of primary importance is the way that litter's pH changes over time. When alum is first added, the litter pH initially drops to approximately pH 5 and then gradually increases to around pH 7 when the flock is removed from the house. Above pH 5, Al phosphate stability decreases greatly, so Al phosphates may initially form and then are dissolved as pH rises over time. Alternatively, the reactivity of Al and phosphate could be kinetically controlled rather than dictated purely by the solubility of minerals. If Al hydroxides precipitation rates are much faster than Al phosphate precipitation rates at 20–30°C and in the presence

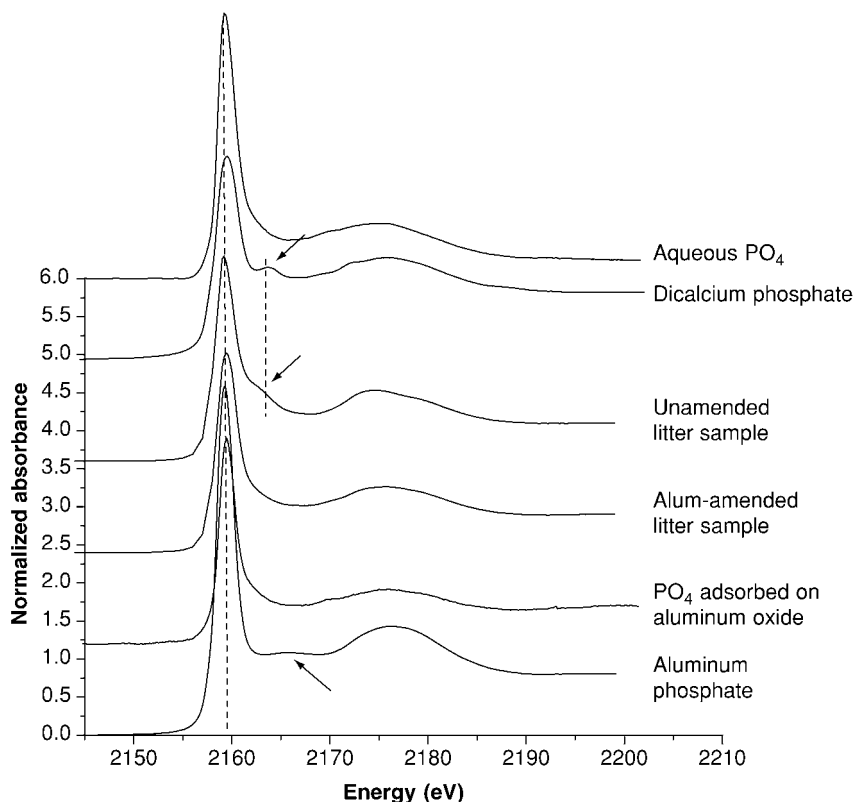


Figure 14 XANES spectra of natural and alum-amended poultry litter taken at P K-edge. Adapted from Peak *et al.* (2002).

of large quantities of organic matter, then Al phosphate formation may be kinetically inhibited. This theory is supported by the observation (Grossl and Inskeep, 1991, 1992; Inskeep and Silvertooth, 1988) that inhibition of Ca phosphate precipitation by organic ligands is responsible for stabilizing thermodynamically instable Ca phosphates in natural systems.

While differences in the amended and unamended litters can be seen, the fingerprinting technique is to some extent relying on the absence of peaks to assign speciation in these litters. To gain confidence in peak assignment, S and Ca XANES was conducted on the samples along with P XANES. Since peaks consistent with dicalcium phosphate were seen in the unamended P XANES spectrum, one would also expect these peaks to appear at the Ca edge. Figure 15 shows the spectra of an unamended and alum-amended poultry litters taken at the S and Ca edge, and compared to various reference compounds. The Ca XANES spectrum of the unamended poultry litter also

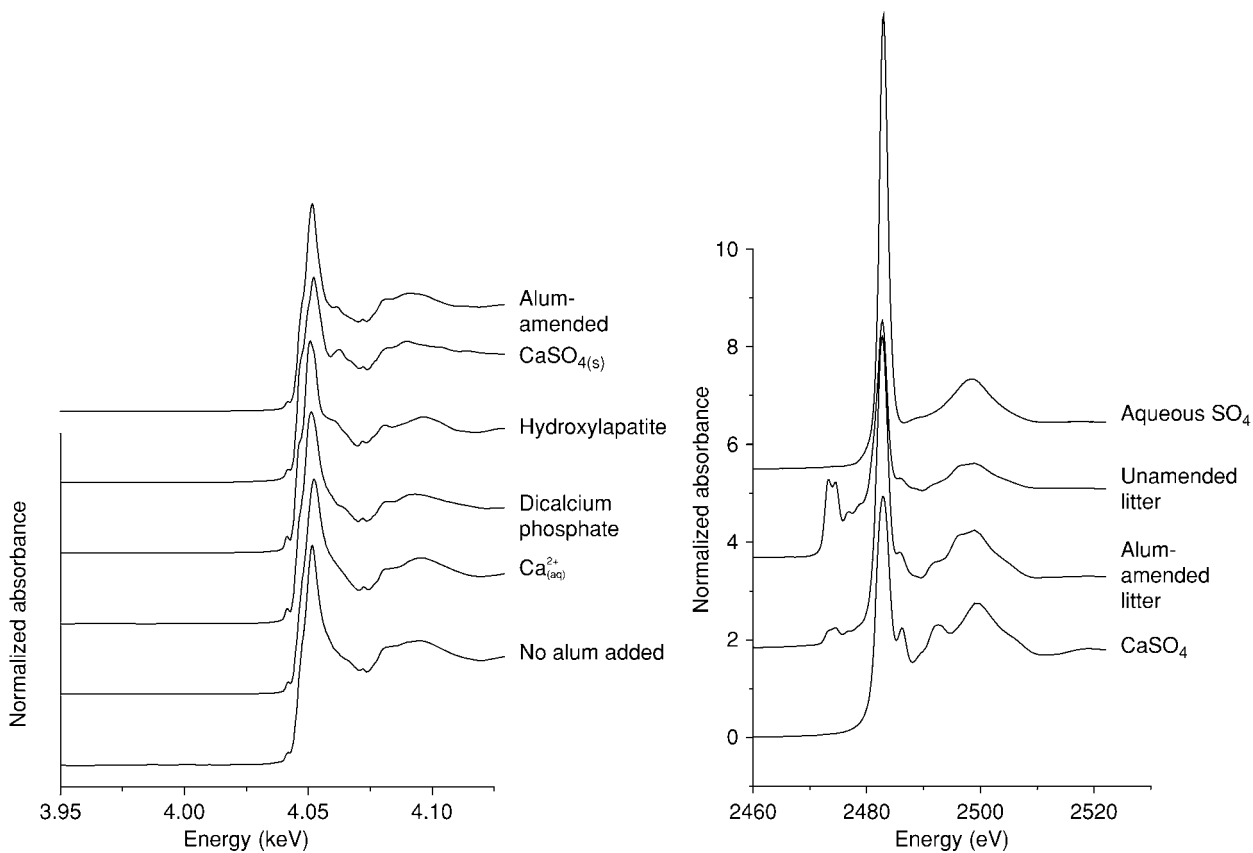


Figure 15 XANES spectra of natural and alum-amended poultry litter sample taken at the S and Ca edge.

seems consistent with dicalcium phosphate, which greatly strengthens the assignment of peaks in the P XANES spectrum. In the amended litter, both Ca and S XANES suggest that gypsum (CaSO_4) is the dominant Ca phase present in the litter.

These experiments clearly demonstrate that XANES spectroscopy is suitable to perform direct chemical speciation of P in organic wastes. However, the results also point out that determination of organic phosphate with XANES is quite limited and that P, S, Ca XANES and solution state NMR studies may all be required to conclusively determine the phosphate chemistry in wastes.

Case Study 2: Correlating Changes in Animal Diets with Changes in Phosphorus Speciation in Poultry Litters and Turkey Manures

Modifying poultry diets by reducing mineral P supplementation and/or adding phytase may change the chemical composition of P in wastes and affect the mobility of P in waste-amended soils. Toor and coworkers (2005c) compared the speciation of P in wastes produced by broiler chickens and turkeys from either normal diets, or diets with reduced amounts of non-phytate P (nPP) and/or phytase, using a combination of chemical fractionation and XANES spectroscopy.

Phosphorus K-edge XANES spectroscopy of all wastes was conducted at the Canadian Synchrotron Radiation Facility (CSRF) at the University of Wisconsin-Madison Synchrotron Radiation Center (SRC) using a double crystal monochromator (DCM) beamline. The CSRF DCM is a UHV beamline, so it was possible to collect both FY and TEY spectra for all samples. The TEY data was used for quantitative analysis because it is not as subject to self-absorption distortions of the spectra. Waste samples were then fit using a variety of P reference standards and the linear combination approach. Principal component analysis was used to determine that four components were needed to describe this data set. The P standards needed to obtain a good fit to the litter and manure samples used in this study were: dicalcium phosphate (monetite phase), hydroxylapatite, phytic acid, and aqueous phosphate. We used the aqueous phosphate standard as an approximation for all “free and weakly bound phosphate” in the system. This encompasses both adsorbed and free aqueous phosphate. This species is expected to correlate strongly with WEP levels. Figure 16 shows the results of performing LC XANES fitting on the turkey manures.

In manures from animals with a normal diet, P is primarily in a dicalcium phosphate form with some phytic acid. As P is removed from the diet, this reduced the WEP in manures and more crystalline apatite phases of Ca phosphate are present. This conversion from more soluble Ca phosphate

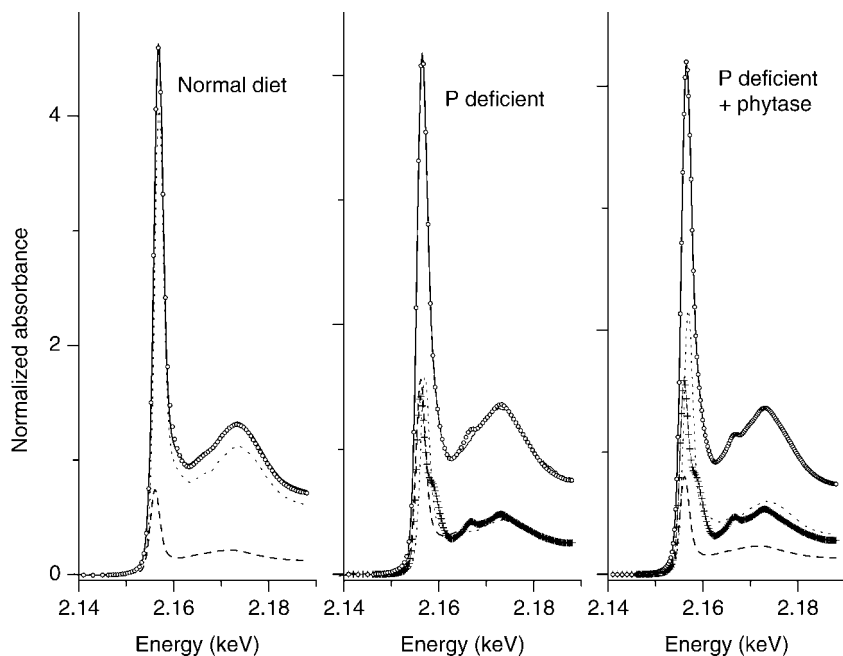


Figure 16 Linear combination fit results for turkey manure samples. The original spectra are shown with a solid line (—), total fit is represented with open circles (○), dicalcium phosphate is represented with a dashed line (---), phytic acid is represented with a dotted line (....), and hydroxylapatite is represented with a plus (+). Of special interest is the appearance of hydroxylapatite in the P deficient as well as P deficient plus phytase samples. The presence of this species can be explained by differences in the Ca to P ratios of these manure samples. From Toor *et al.* (2005c).

phases to low solubility apatite phases results in a large decrease in WEP from both the P deficient and P deficient plus phytase manures. This is a general trend that can be observed by plotting the results from LC XANES fitting versus (i) total Ca to P ratio or (ii) $\text{H}_2\text{O-P}$ in the wastes (Fig. 17).

The relationship between different chemical species (from XANES analysis) and total Ca to P ratio and $\text{H}_2\text{O-P}$ levels for the different manure and litter samples suggests that the litter or manure Ca to P ratio is an extremely useful parameter in estimating the solubility of Ca phosphate minerals in these samples. At total Ca to P ratios less than 1.7 in wastes, aqueous phosphate can be observed in the broiler litter and normal turkey manure samples and dicalcium phosphate is the only mineral phase present. At total Ca to P values of greater than 1.7 (turkey manures), however, hydroxylapatite can be observed and aqueous phosphate contributions disappear. Hydroxylapatite is far

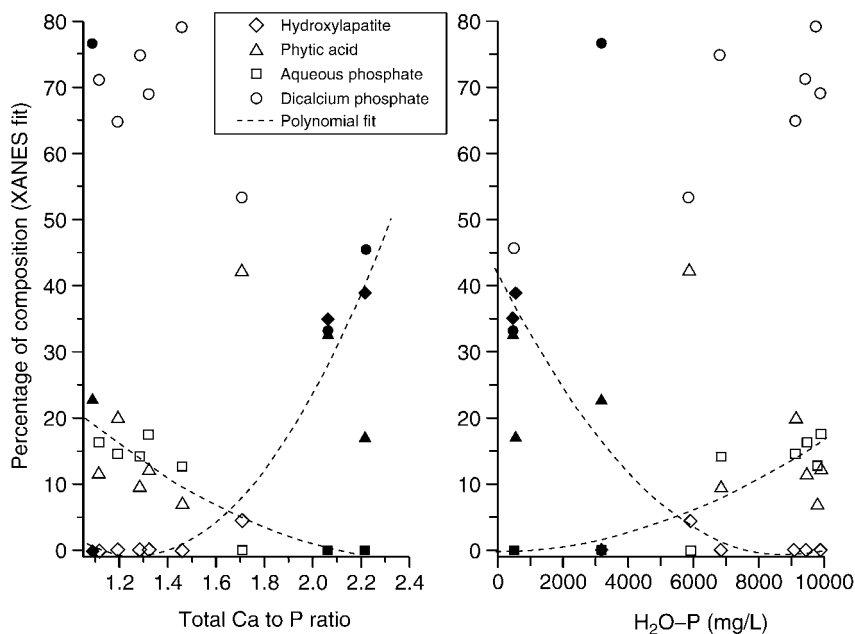


Figure 17 Relationships of total Ca to phosphorus ratio and H₂O-P (extraction ratio of 1 to 200) with P forms determined with XANES spectroscopy for broiler litters and turkey manures. Turkey manures are indicated by gray data points while broiler litters are open symbols. Adapted from Toor *et al.* (2005c).

less soluble than dicalcium phosphate, so when this phase controls P solubility then solution P levels are expected to accordingly be decreased. When the more soluble dicalcium phosphate phase controls solubility, more aqueous phosphate will be present in the litters and manures. The XANES results are strengthened by the fact that it is well known that Ca to P ratio affects the stable form of Ca phosphate. For example, at Ca to P ratios of 1.0 dicalcium phosphate dominates, at Ca to P ratios of 1.5 tricalcium phosphate is dominant, and at Ca to P ratios of 1.67 and above hydroxylapatite is the stable phase (Ben-Nissan *et al.*, 1995).

Comparison of the Scope and Limitations of Nuclear Magnetic Resonance and X-Ray Absorption Near Edge Structure Spectroscopy

Nuclear Magnetic Resonance and XANES spectroscopic methods are both element-specific and sensitive to the direct average chemical environment of the

atom. Both are also ideally suited for the analysis of heterogeneous materials. But while solid state NMR requires drying and grinding of the sample, XANES spectroscopy can be performed on the moist, unaltered sample and allows for true *in situ* conditions. Despite this difference, both methods generally agree with each other and provide complementary information about P compounds in organic wastes.

Further advantages of XANES over NMR spectroscopy are the better sensitivity and the fact that XANES can be performed with every element, given the availability of an x-ray beamline with the adequate energy range. On the other hand, NMR works well with a small number of elements (H, C, P, F, B, Al) and at greatly reduced sensitivity with others (e.g., N, Si) (Drago, 1992). Using XANES spectroscopy thus allows for the efficient analysis of not only P species, but also of other elements in wastes, such as Ca and S, or the transition metals. For example, poultry litter contains large amounts of Ca (Jackson *et al.*, 2003; Moore *et al.*, 1995), which plays an important role in determining the P species in litter. Transition metal levels are generally lower but also form solid phosphate phases that can control P speciation. Solid phosphate phases involving transition metal cations with unpaired electrons can, however, not be analyzed with NMR because of paramagnetic line broadening (Blumberg, 1960; Sutter *et al.*, 2002).

The advantage of NMR spectroscopy, on the other hand, is that the better resolution allows for a more precise identification and quantification of P species, as can be illustrated by the comparison of the work of Hunger *et al.* (2004) with that of Peak *et al.* (2002).

V. SUMMARY

Of the various methods available to characterize P forms in organic wastes, the choice of a particular method will depend on the objectives of the study and the ease of access to some of the instruments such as NMR and XANES. From an agronomic perspective, only the total P concentration has been historically considered important to calculate the annual P application rates. But this method is very poorly linked to the true amount of P that is available to plants and is susceptible to P loss. A simple extraction with water may provide information about the nature of inorganic P and easily degradable organic P compounds, which can more accurately be used to compare the relative solubility of P in the wastes.

Information about detailed P forms in the wastes can be obtained by sequential chemical fractionation methods. The main disadvantage with

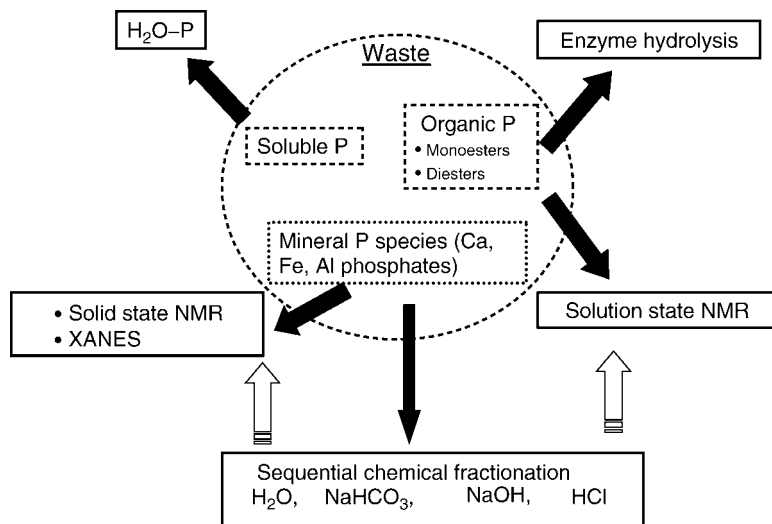


Figure 18 Summary of methods for phosphorus analysis in organic wastes.

chemical fractionation is there is no confirmation if a particular extractant is actually solubilizing the target P form. However, the studies involving use of NMR and XANES in combination with chemical extraction have greatly enhanced our understanding of various P compounds (Fig. 18). For example, the first fractions extracted in sequential P fractionation are H₂O and NaHCO₃ extractable P, both of which contain orthophosphate and easily degradable organic P such as labile monoesters, phospholipids, and DNA (Turner and Leytem, 2004). Other P fractions, such as NaOH and HCl extractable, are less soluble and contain more recalcitrant forms of P such as phytic acid and hydroxylapatite (Toor *et al.*, 2005c). Spectroscopic techniques such as NMR and XANES are excellent tools to characterize P in wastes, however, these are expensive and require experienced spectroscopists. Therefore, these have limited applicability for adoption. However, a combination of chemical fractionation and spectroscopic techniques will help to elucidate the nature of P species in wastes. Having confirmed the different P species, the sequential chemical fractionation method can be simplified. This will improve accuracy and confidence in use of these fractionation schemes. Similarly, the other chemical P fractionation scheme (Barnett, 1994b), which fractionates P into organic P species can be validated with spectroscopic analysis (e.g., NMR) or commercial phosphatase enzymes.

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WHEAT GENETICS RESOURCE CENTER: THE FIRST 25 YEARS

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The Wheat Genetics Resource Center, a pioneering center without walls, has served the wheat genetics community for 25 years. The Wheat Genetics Resource Center (WGRC) assembled a working collection of over 11,000 wild wheat relatives and cytogenetic stocks for conservation and use in wheat genome analysis and crop improvement. Over 30,000 samples from the WGRC collection of wheat wild relatives, cytogenetic stocks, and improved germplasm have been distributed to scientists in 45 countries and 39 states in the United States. The WGRC and collaborators have developed standard karyotypes of 26 species of the *Triticum/Aegilops* complex, rye, and some

perennial genera of the Triticeae. They have developed over 800 cytogenetic stocks including addition, substitution, and deletion lines. The anchor karyotypes, technical innovations, and associated cytogenetic stocks are a part of the basic tool kit of every wheat geneticist. They have cytogenetically characterized over six-dozen wheat–alien introgression lines. The WGRC has released 47 improved germplasm lines incorporating over 50 novel genes against pathogens and pests; some genes have been deployed in agriculture. The WGRC hosted over three-dozen scientists especially from developing countries for advanced training. The WGRC was engaged in international agriculture through several collaborating projects. Particularly noteworthy was the collaborative project with Centro Internacional de Mejoramiento de Maiz y Trigo (CIMMYT) on the production of synthetic wheats. It is estimated that “by the year 2003–2004, 26% of all new advanced lines made available through CIMMYT screening nurseries to cooperators for either irrigated or semi-arid conditions were synthetic derivatives.” The WGRC is applying genomics tools to further expedite the use of exotic germplasm in wheat crop improvement.

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I. INTRODUCTION

“A cytogeneticist is one who curates the genome of an organism, identifies and maintains gene inventories, and delineates a genetic road map. A genetic engineer who constructs exotic stocks for basic and applied research applications. Above all, he is the one person you always get in touch with when you need information and a genetic stock at a moment’s notice!”

Bikram Gill, circa 1982

This is an old-fashioned definition of a cytogeneticist in the tradition of Barbara McClintock, Charley Burnham, and Marcus Rhoades of maize; Ernie Sears of wheat; and Charley Rick of tomato, all tracing their pedigrees to E. M. East at the Bussey Institution of Harvard University. The senior author had the privilege of working as a graduate student with Charley Rick, as a postdoctoral fellow with Ernie Sears, and with Charley Burnham on a collaborative project on developing a chromosomal translocation tester set in tomato (Gill *et al.*, 1980). Rick made frequent trips to South America to collect wild tomato species, ran a gene bank, conducted interspecific hybridization and breeding research, constructed cytogenetic maps, and published the *Tomato Genetic Coop*. Ernie Sears (1954) developed wheat aneuploid stocks to genetically dissect and engineer the polyploid genome of wheat. The senior author also had the fortune of working on wild wheats at the University of California, Riverside, with Giles Waines and Lennert Johnson.

In 1979, on his way to a cytogenetics position at Kansas State University, which is located in one of the largest wheat-growing regions in the world, he stopped to see Ernie Sears in Missouri. His advice, “Kansas has a great breeding program, but they need basic genetics research to complement it.” Of the current research team, coauthor Rollie Sears joined as a wheat breeder in 1980 to be replaced by Allan Fritz in 2000; John Raupp and Duane Wilson joined as research assistants in 1980 and 1984, respectively; Stan Cox as a USDA Research Geneticist in 1984 (to be replaced by Gina Brown-Guedira in 1997); and Bernd Friebe as a research cytogeneticist in 1989.

A two-pronged wheat-research program was initiated. Molecular cytogenetic studies were conducted on “Chinese Spring,” which was used by Ernie Sears to develop aneuploid stocks and accepted as the international model for wheat genetics and polyploidy research. Following the groundbreaking work on the cytogenetic identification of individual chromosomes of “Chinese Spring” wheat (Gill and Kimber, 1974b), a standard karyotype and nomenclature system for wheat chromosomes was developed with the hope that it would “... vastly expand our ability to cytogenetically analyze and manipulate the genome of wheat with unprecedented precision and efficiency” (Gill *et al.*, 1991a). Around the same time, we initiated a collaborative research program with Dr T. R. Endo, then at Nara University, Japan, funded by the Japanese Society for the promotion of Science and the National Science Foundation (NSF) and United States Department of Agriculture National Research Initiative (USDA–NRI) in the United States to develop more than 400 true-breeding deletion stocks in wheat (Endo and Gill, 1996). These deletion stocks were used to develop cytogenetically based, physical maps of molecular markers for the 21 chromosomes of wheat and revealed the nonrandom distribution of genes and recombination along the chromosome length (Delaney *et al.*, 1995b,c; Gill and Gill, 1994; Gill *et al.*, 1993, 1996a,b; Hohmann *et al.*, 1994; Kota *et al.*, 1993; Michelson-Young *et al.*, 1995; Werner *et al.*, 1992a). Sixteen thousand expressed sequence tag (EST) loci were mapped in deletion bins [Qi *et al.*, 2003, 2004; see also *Genetics* (2004), Special Section: Wheat, **168**, 583–712] and compared to the sequenced genome of rice to construct *in silico* wheat maps (<http://www.tigr.org/tdb/e2k1/tael/>) that are driving gene discovery in wheat. The international wheat genetics community is now embarked on a project to sequence the gene space of wheat (Gill *et al.*, 2004). Such a project would have a huge impact on wheat-crop improvement and investigations of polyploidy and its role in genome evolution, speciation, and plant productivity, as most crop plants that feed us are polyploids.

In the second research thrust, we assembled a working collection of wild wheat species for conservation, evaluation, and utilization in germplasm enhancement and broadening the gene pool of wheat. In the beginning, we chose the hard red winter wheat cultivar “Wichita,” released in the 1940s as the recipient wheat parent. This cultivar was a research model in

which Rosalind Morris (University of Nebraska, Lincoln) had developed a complete set of monosomic stocks. We screened a small collection of *Ae. tauschii*, the D-genome donor of bread wheat, and discovered an abundance of resistance to the most virulent races of Hessian fly (Hatchett and Gill, 1981), a highly destructive pest of wheat. Additional screening against leaf rust and other pathogens revealed many single accessions harboring multiple resistance factors against a number of pathogens and pests (Gill *et al.*, 1986b). We used embryo culture to obtain direct hybrids between “Wichita” and *Ae. tauschii*, and recovered highly desirable and homozygous resistant lines in BC₂F₂ families (Gill and Raupp, 1987), and the first germplasm release was made in 1985 (Gill *et al.*, 1986a). However, it was soon clear that wheat breeders had little interest in “Wichita”-based germplasm. It was not until Stan Cox joined the team and began germplasm-enhancement research with advanced breeding materials in close collaboration with Rollie Sears and the Great Plains wheat breeders that the chasm between basic genetics and breeding work was bridged, and the worldwide impact of the new genetics on wheat varietal improvement programs was achieved as documented here (Cox, 1991, 1998).

In 1984, the Wheat Genetics Resource Center (WGRC) was formally recognized as a center of excellence at Kansas State University with a more secure funding base. The WGRC has been a pioneering center without walls, conducting interdisciplinary and interdepartmental, collaborative, team-oriented research involving K-State and USDA–ARS scientists, locally, and others, nationally and internationally. The hallmarks of the WGRC mission have been full integration of basic and applied research and service to the wheat community as a “one-stop shop” for the free sharing of genetic materials, technical know how, and knowledge through research publications, distribution of germplasm, and training of graduate students, post-doctoral fellows and visiting scientists, especially from developing countries through workshops, hands-on research in WGRC laboratories, and long-term collaborative research projects (for details, see www.ksu.edu/wgrc). The aim of this review is to briefly highlight WGRC research in wheat genetic resources, cytogenetics, genomic breeding, chromosome engineering, and germplasm development, its impact, and the future outlook.

II. WHEAT GENETIC RESOURCES

A. TAXONOMIC CONSIDERATIONS

Wheat belongs to the grass tribe Triticeae, which contains approximately 350 species and 13–26 genera. More than 75% of the species are perennial and many are used as forage crops. Annual Triticeae species include wheat,

barley, and rye; all agriculturally important grasses either for human or animal consumption.

Twenty-six classification schemes for wheat and wheat relatives have been proposed since 1917. These early classification systems were based on morphological characteristics. Historically, the genus *Triticum* was treated separately from the genus *Aegilops* although several authors have grouped the two together in one large genus based on cytogenetic evidence. Kihara (1954) was the first to use a genomic treatment and his proposed genome symbols are still in use. Eleven classification systems are accepted, three address only the genus *Triticum* and two only the genus *Aegilops*. Only Kimber and Sears (1987) and Kimber and Feldman (1987) following Bowden (1959) treat all species under the genus *Triticum*. The remaining classification systems support the widely accepted idea of two separate genera, *Triticum* and *Aegilops*. In this publication, we will use the most recent classification proposed by van Slageren (1994). Descriptions of all the historical and current classification systems can be seen at <http://www.ksu.edu/wgrc/Taxonomy/taxintro.html/>.

B. COLLECTION AND MAINTENANCE

The world collection of *Triticum* and *Aegilops* consists of approximately 17,500 accessions distributed in a dozen or so gene banks worldwide (<http://www.singer.cgiar.org/>). Data previously available only in the literature, through gene bank records or by personal communication can now be accessed via the Internet. The working collection maintained by the WGRC consists of 3119 accessions comprising annual *Triticum* and *Aegilops* species and are listed in Table I. This working collection is a composite, as distinguished from core collections established by pioneering plant explorers. The entries in the germplasm collection are from expeditions by the University of Kyoto (Japan) in 1955, 1959, 1966, and 1970; Johnson and coworkers (University of Riverside, CA, USA) 1966, 1972, and 1973; E. Nevo and colleagues (University of Haifa, Israel); and R. J. Metzger (University of Oregon, Corvallis, USA), J. Hoffman (USDA-ARS), G. Kimber (University of Missouri, Columbia, USA), S. Jena (University of Saskatchewan, Canada), and A. Sencor, M. Kanbertay, and C. Tüten (Aegean Agricultural Research Institute, Menemen, Izmir, Turkey), 1979, 1984, and 1985. Additional accessions from major gene banks of the world include ICARDA (Aleppo, Syria), the USDA Small Grains Collection (Aberdeen, ID, USA), the N.I. Vavilov Institute (St. Petersburg, Russia), and the Institute for Genetics and Crop Plant Research (Gatersleben, Germany).

Table I
Composition of the WGRC Gene Bank, 2005 (Genome Symbols in Parentheses)

Species	Number of accessions
Diploid ($2n = 14$) species	
<i>T. monococcum</i> L. (A^m)	600
<i>T. urartu</i> Tumanian ex Gandilyan (A^u)	173
<i>Ae. bicornis</i> (Forssk.) Jaub. & Spach (S^b)	12
<i>Ae. caudata</i> L. (C)	18
<i>Ae. comosa</i> Sm. In Sibth. & Sm. (M)	20
<i>Ae. longissima</i> Schweinf. & Muschl. (S^l)	9
<i>Ae. mutica</i> Boiss. (T)	10
<i>Ae. searsii</i> Feldman & Kislev ex Hammer (S^s)	18
<i>Ae. sharonensis</i> Eig (S^{sh})	9
<i>Ae. speltooides</i> Tausch (S)	92
<i>Ae. tauschii</i> Coss. (D)	528
<i>Ae. umbellulata</i> Zhuk. (U)	46
<i>Ae. uniaristata</i> Vis. (N)	20
<i>H. villosa</i> (L.) Schur (V)	94
Polyloid tetraploid ($2n = 28$) and hexaploid ($2n = 42$) <i>Triticum</i> and <i>Aegilops</i> species.	
<i>T. timopheevii</i> Zhuk. (A^tG)	295
<i>T. turgidum</i> L. (AB)	488
<i>T. aestivum</i> L. (ABD)	301
<i>Ae. biuncialis</i> Vis. ($U^{bi}M^{bi}$)	36
<i>Ae. columnaris</i> Zhuk. ($U^{co}X^{co}$)	11
<i>Ae. crassa</i> Boiss ($4x (X^{cr}D^{cr1}), 6x (X^{cr}D^{cr1}D^{cr2})$)	27
<i>Ae. cylindrica</i> Host (C^cD^c)	42
<i>Ae. geniculata</i> Roth (U^gM^g)	139
<i>Ae. juvenalis</i> (Thell.) Host ($X^jD^jU^j$)	9
<i>Ae. kotschy</i> Boiss. (U^kS^k)	18
<i>Ae. neglecta</i> Req. ex Bertol (U^nX^n and $U^nX^nN^n$)	66
<i>Ae. peregrina</i> (Hack. in J. Fraser) Marie & Weiller (U^pS^p)	29
<i>Ae. triuncialis</i> L. (U^tC^t)	183
<i>Ae. vavilovii</i> ($X^{va}S^{va}S^{va}$)	8
<i>Ae. ventricosa</i> Tausch (N^vD^v)	16
<i>T. zhukovskyi</i> Menabde & Ericz (A^tAG)	1
Genetic stocks	
<i>Ae. tauschii</i> synthetic and parental lines	311
Alien addition	371
Alien substitution	251
Alloplasmic	8
Amphiploid/partial amphiploid	121
Aneuploid	299
Deletion/duplication/deficiency	421
Germplasm	75

(continued)

Table I (continued)

Species	Number of accessions
Mutant/Marker	401
Mapping and RIL populations (44 populations)	5,551
Substitution	149
Translocation	143
Transgenic	33
TOTAL	11,497

For evolutionary relationships between the *Triticum* and *Aegilops* species, see Fig. 1.

Samples in the working collection are maintained at 40 °F (4 °C) and 25% RH. Seed is stored in moisture-proof, heat-sealable pouches from the Kapac Corporation (Minneapolis, MN). These pouches are trilaminate (50 GA PET/0.00035 foil/3 mil LLDPE) and form a nearly impervious barrier against humidity. In addition, a 5-g packet of silica gel is included in the pouch as a desiccant. Whenever seed is removed from a pouch, the silica gel packet is checked and replaced if necessary. When the number of seed in a line drops below 100, five plants are grown to ensure an adequate supply. At that time, additional pest resistance screening may be done and/or the lines checked for proper species identification.

Long-term storage of seed in a freezer is maintained at -20°C. Seed samples are stored in the same moisture-proof, heat-sealable pouches as the samples in the working collection. Under these conditions, seed viability should approach 50 years. This permanent collection was started in 1991 and is housed off-site at the Kansas Crop Improvement Association in Manhattan, KS.

Detecting duplicate accessions and identifying geographical areas where the germplasm collections are lacking is a foremost priority. We have completed a search of available databases via the Internet and using a world collection database established by ICARDA, Aleppo, Syria. Database development and coordination among gene banks will reduce duplication of research and promote the utilization of germplasm resources (Raupp *et al.*, 1997). When we detect a potential duplication, storage protein profiles are used for genetic confirmation. We are making a concerted effort to obtain complete and accurate passport data on the accessions with the help of other researchers and gene bank coordinators. Database searches also helped with missing collection data. The policy of the WGRC now is to provide this valuable passport data when seed is requested.

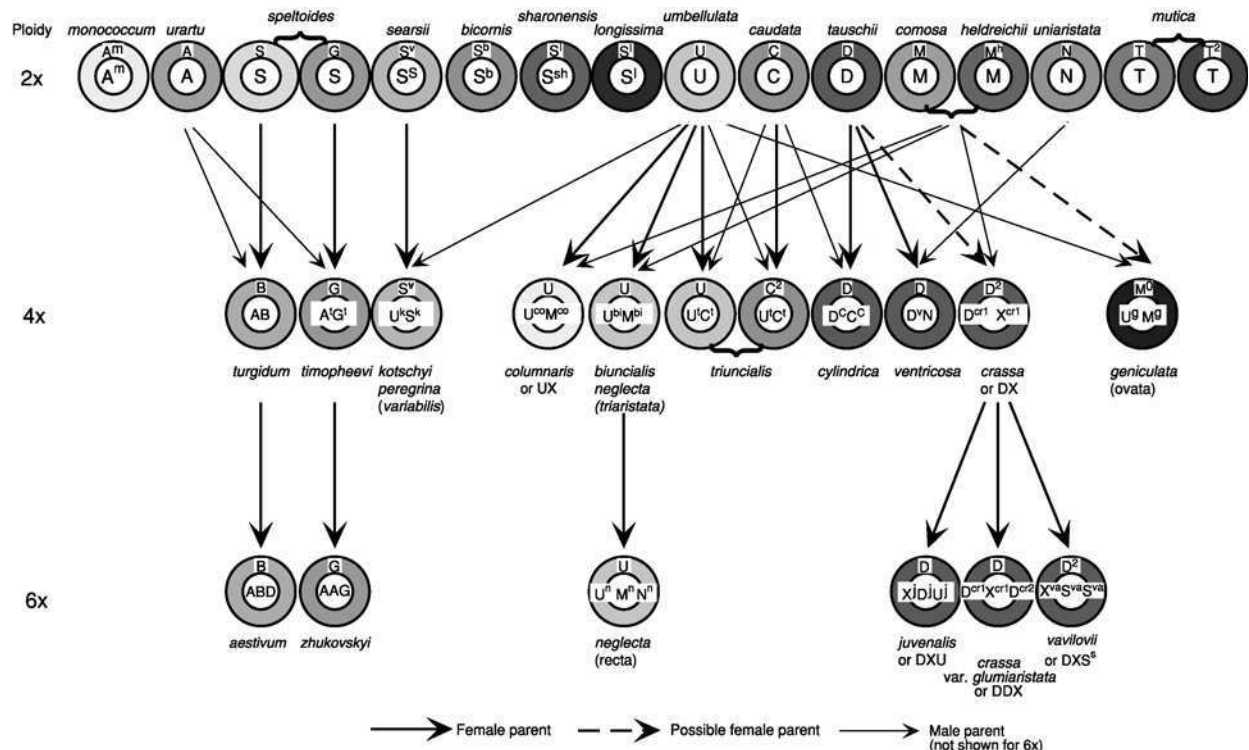


Figure 1 The *Triticum* and *Aegilops* genera contain 13 diploid, 14 tetraploid, and 6 hexaploid species and are a classic example of speciation by allopolyploidy. The polyploids constitute the A-genome cluster, which includes wild and cultivated wheat, the U-genome cluster, and the D-genome cluster. The knowledge of evolutionary relationships is important for their use in wheat crop improvement. This figure is modified with permission from the author [Tsunewaki, K. (1996). Plasmon analysis as a counterpart of genome analysis. In "Methods of Genome Analysis in Plants" (P. P. Jauhar, Ed.). Reprinted with permission of CRC Press, Boca Raton, FL].

Several researchers (Chapman, 1985; Croston and Williams, 1981; Holubec *et al.*, 1992) have identified priority areas for wheat germplasm collection. Researchers near the priority areas hopefully can, or have, filled these gaps, permitting germplasm exchange with cooperating gene banks. We have completed such surveys for *Ae. speltooides*, *Ae. tauschii*, and *Haynaldia villosa* (L.) Schur and hope to complete the same for other species in the WGRC gene bank.

The WGRC has established contacts with curators in Japan, Germany, the Russian Federation, Syria, and elsewhere to promote joint studies and sharing of germplasm. Under our visiting scientist program, we invite scientists from these and other countries for joint research. Our aim is to establish the world's most comprehensive "working collection" of wild wheats and promote basic and applied research on this collection, and on conservation and utilization of the world's germplasm of wheat.

C. EVALUATION AND GENETIC DIVERSITY ANALYSIS OF THE WGRC COLLECTION

The species collection, hybrid derivatives, amphiploids, and addition and translocation lines are intensely evaluated for useful genetic variation by national and international research collaborators. A large number of accessions from the germplasm collection have been evaluated for host plant resistance to leaf rust (*Puccinia triticina* Eriks.), stem rust (*Puccinia graminis* Pers. f. sp. *tritici* Eriks. & Henn.), yellow rust (*Puccinia striiformis* West.), Karnal bunt (*Tilletia indica* Mit.) [= *Neovossia indica* (Mit.) Mund.], leaf blotch [*Stagonospora nodorum* (Berk.) Castellani & E. G. Germano] [= *Septoria nodorum* (Berk.) Berk. in Berk. & Broome], scab or head blight (*Fusarium graminearum* Schwabe), tan spot [*Drechslera tritici-repentis* (Died.) Shoem.], powdery mildew [*Blumeria graminis* (DC.) E. O. Speer] (= *Erysiphe graminis* DC. ex Merat), wheat streak mosaic virus, barley yellow dwarf virus, Hessian fly (*Mayetiola destructor* Say), greenbug (*Schizaphis gramineum* Rondani), Russian wheat aphid (*Diuraphis noxia* Mordvilko), and wheat curl mite (*Eriophyes tulipae* Kiefer). The screening of wild wheat germplasm is ongoing and continuously documented (Brown-Guedira *et al.* 1996b, 2002; Cox *et al.*, 1992a; Deol *et al.*, 1995; Gill *et al.* 1983, 1985, 1986b; Lubbers *et al.*, 1991; Malik *et al.*, 2003b; Raupp *et al.* 1988, 1995; Smith *et al.*, 2004; Stoddard *et al.*, 1987).

D. DISTRIBUTION OF THE COLLECTION

Even though there were already extensive collections of wild relatives in the United States and elsewhere by the 1950s, there were only a few sporadic instances of their use in breeding programs. From its very inception, the

WGRC has actively sought to promote the use of wild wheat relatives (the diploid and tetraploid donor species) in broadening the genetic base of cultivated wheat by free sharing of germplasm at no charge and free of Intellectual Property Rights (IPR) (Fig. 2). The WGRC even played a more critical role in increasing use of wild species germplasm by documenting tremendous genetic diversity in the collection and by demonstrating its rapid transfer to wheat by using routine embryo rescue and cytogenetic methods. This naturally created a lot of excitement and as can be seen in Fig. 2, by 1986 there was a large increase in the number of requests for wild species germplasm. That year, the senior author was on a sabbatic leave in Australia, and, as a result of these contacts, the Commonwealth Scientific and Research Organization (CSIRO) began a large project on the use of *Ae. tauschii* in wheat improvement. We also worked with Centro Internacional de Mejoramiento de Maiz y Trigo (CIMMYT) to focus on wheat progenitor species in their wide-crossing program rather than perennial grasses as had been the case until then. Another category of germplasm, cytogenetic stocks, also have seen steady increase in demand, especially the deletion stocks spurred by their use in genome-mapping projects. By 2004, we had distributed 30,222 samples (1461 requests) of seed to scientists in 45 countries and 39 states in the United States. Of these, 18,952 were for cytogenetic stocks, 10,080 for wild wheats, and 1461 for improved germplasm. The number of requests for improved germplasm is an underestimate as it does not include data from the USDA who also distributed samples of seed. Whereas the WGRC has distributed germplasm free of cost until now, with tight budgets and increased demands for germplasm, cost recovery may be an option that needs to be explored in the near future.

III. ADVANCES IN MOLECULAR CYTOGENETICS OF WHEAT AND TRITICEAE SPECIES

“Molecular cytogenetics may be defined as instant cytological, genetic, phylogenetic, and molecular mapping of chromosomes *in situ*” (Gill, 1995). Molecular cytogenetic identification of individual chromosomes is essential for analyzing the biological aspects of chromosome structure, function, evolution, and engineering the genome for crop improvement. Wheat has served as a model system for plant molecular cytogenetics research beginning in the 1970s (see reviews by Faris *et al.*, 2002; Friebe and Gill, 1995; Gill, 1993, 1995; Gill and Friebe, 1998, 2002; Gill and Sears, 1988; Jiang and Gill, 1994b). Each wheat chromosome is divisible into biologically meaningful heterochromatic and euchromatic regions (Gill *et al.*, 1991a). Many DNA sequences when used as probes in conjunction with *in situ*

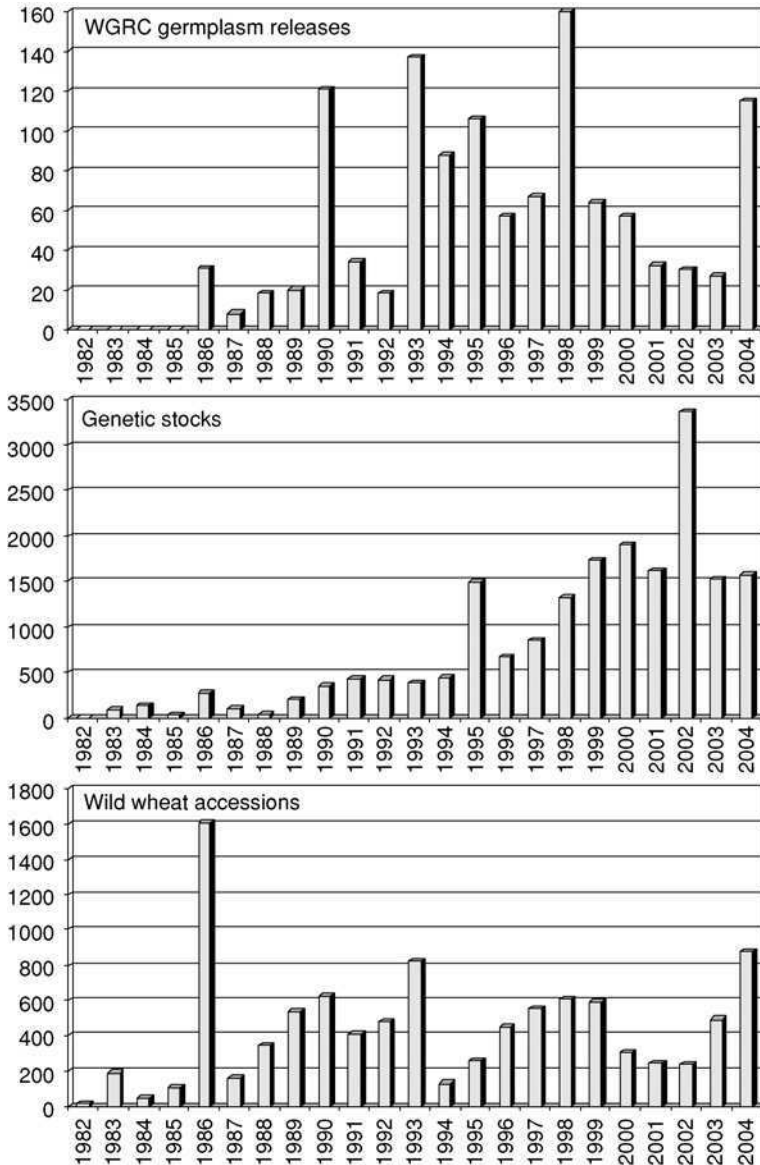


Figure 2 Requests for wheat genetic materials from 1982 through 2004.

hybridization provide further opportunities for molecular karyotyping (Rayburn and Gill, 1985, 1987) and genome painting (Lapitan *et al.*, 1986; Zhang *et al.*, 2004b). Molecular descriptors have been developed for

cytogenetic landmarks such as heterochromatin (Badaeva *et al.*, 1996a; Rayburn and Gill, 1986), nucleolus organizer regions (Badaeva *et al.*, 1996b; Jiang and Gill, 1994a; Mukai *et al.*, 1990, 1991), centromeres (Jiang *et al.*, 1996; Zhang *et al.*, 2001, 2004a), subtelomeric regions (Li *et al.*, 2004; Zhang *et al.*, 2004a), and telomeres (Friebe *et al.*, 2001; Werner *et al.*, 1992b).

Initially, telocentric chromosomes were C-banded to cytogenetically identify the 21 chromosomes belonging to the A, B, and D genomes of hexaploid wheat “Chinese Spring” (Gill and Kimber, 1974b; Gill *et al.*, 1991a). The chromosomes of AB-genome tetraploids, and the A- and D-genome diploids were constructed based on comparative banding analysis with the ABD genome of wheat (Friebe and Gill, 1995). The A¹G-genome chromosomes of *T. timopheevii* subsp. *timopheevii* were cytogenetically identified based on their pairing affinities with wheat telocentrics and the C-banding analysis of paired chromosomes (Badaeva *et al.*, 1994; Chen and Gill, 1983; Gill and Chen, 1987). The chromosomes of related Triticeae species with different genomes were cytogenetically identified by C-banding analysis of alien chromosome additions to “Chinese Spring” wheat as first demonstrated in rye (Gill and Kimber, 1974b; Mukai *et al.*, 1992).

The knowledge and biological resources of the model wheat genome and other characterized basic Triticeae genomes can in turn be used to determine the cytogenetic and phylogenetic affinity of individual chromosomes of the other alien taxa. This was demonstrated in the analysis of the genome structure of *Ae. cylindrica* (Linc *et al.*, 1999). The C- and D-genome chromosomes of *Ae. cylindrica* were identified by comparative C-banding and fluorescent *in situ* hybridization (FISH) with D-genome specific DNA sequences. The karyotypes of other D-genome and U-genome cluster diploid and polyploid species have been similarly analyzed (Badaeva *et al.*, 2002, 2004). Standard karyotypes have been developed for all the species of *Triticum* and *Aegilops*, and certain species of *Secale*, *Haynaldia*, *Agropyron*, *Elymus*, and *Hordeum* (Table II). The standard karyotypes allow cytogenetical monitoring of the transfer of alien chromosome segments during wide hybridization (Friebe *et al.*, 1996b; Jiang and Gill, 1994a).

IV. GENOMIC BREEDING AND INTERGENOMIC TRANSFERS BY CHROMOSOME ENGINEERING

A. THE JOURNEY FROM GENOME SHARING TO GENE DONORS

Between 1918 and 1925, Sakamura (1918) and his student Kihara (1919) at Hokkaido University, Japan, and Sax (1922) at Harvard University reported their classic studies on the genetic architecture of the various

Table II
Standard Karyotypes and Cytogenetic Identification of Individual Chromosomes, Deletion, Addition, or Substitution Lines of *Triticum*, *Aegilops*, and other Triticeae Species in Wheat Characterized and Maintained by the WGRC

Species	Ploidy level (2n)	Genome formula	Chromosome addition lines	Telosomic addition lines	Substitution lines	Reference
<i>Ae. bicornis</i>	2x	S ^b				Badaeva <i>et al.</i> , 1996a,b; Friebe and Gill, 1995
<i>Ae. biuncialis</i>	4x	U ^{bi} M ^{bi}				Badaeva <i>et al.</i> , 2004
<i>Ae. caudata</i>	2x	C	6		1	Badaeva <i>et al.</i> , 1996a,b; Friebe <i>et al.</i> , 1992c
<i>Ae. columnaris</i>	2x	U ^{co} X ^{co}				Badaeva <i>et al.</i> , 2004
<i>Ae. comosa</i>	4x	M	1			Badaeva <i>et al.</i> , 1996a,b; Friebe <i>et al.</i> , 1996a; Nasuda <i>et al.</i> , 1998
<i>Ae. crassa</i>	4x	X ^{cr} D ^{cr1}				Badaeva <i>et al.</i> , 1998, 2002
<i>Ae. crassa</i>	6x	X ^{cr} D ^{cr1} D ^{cr2}				Badaeva <i>et al.</i> , 1998, 2002
<i>Ae. cylindrica</i>	4x	C ^c D ^c	1	1		Endo and Gill, 1996, Linc <i>et al.</i> , 1999
<i>Ae. geniculata</i>	4x	U ^g M ^g	14	11		Friebe <i>et al.</i> , 1999a
<i>Ae. juvenalis</i>	6x	X ^j D ^j U ^j				Badaeva <i>et al.</i> , 2002
<i>Ae. kotschyi</i>	4x	U ^k S ^k				Badaeva <i>et al.</i> , 2004
<i>Ae. longissima</i>	2x	S ^l	7 + 7 + 1	14	43	Badaeva <i>et al.</i> , 1996a,b; Friebe <i>et al.</i> , 1993c
<i>Ae. mutica</i>	2x	T	B			Badaeva <i>et al.</i> , 1996a,b; Friebe <i>et al.</i> , 1995b, 1996a
<i>Ae. neglecta</i>	4x	U ⁿ X ⁿ				Badaeva <i>et al.</i> , 2004
<i>Ae. neglecta</i>	6x	U ⁿ X ⁿ N ⁿ				Badaeva <i>et al.</i> , 2004
<i>Ae. peregrina</i>	4x	U ^p S ^p	14	26		Friebe <i>et al.</i> , 1996c

(continued)

Table II (continued)

Species	Ploidy level (2n)	Genome formula	Chromosome addition lines	Telosomic addition lines	Substitution lines	Reference
<i>Ae. searsii</i>	2x	S ^s	7	14	50	Badaeva <i>et al.</i> , 1996a,b; Friebe <i>et al.</i> , 1995d
<i>Ae. sharonensis</i>	2x	S ^{sh}	10 × 1			Badaeva <i>et al.</i> , 1996a,b; Friebe and Gill, 1995; Friebe <i>et al.</i> , unpublished
<i>Ae. speltoides</i>	2x	S	7 + B	7	6	Badaeva <i>et al.</i> , 1996a,b; Friebe and Gill, 1995; Friebe <i>et al.</i> , 2000b, unpublished
<i>Ae. tauschii</i>	2x	D	7 (in durum wheat)	7		Badaeva <i>et al.</i> , 1996a,b, 2002; Dhaliwal <i>et al.</i> , 1990; Friebe <i>et al.</i> , 1992a
<i>Ae. triuncialis</i>	4x	U ^t C ^t				Badaeva <i>et al.</i> , 2004
<i>Ae. umbellulata</i>	2x	U	6	9		Badaeva <i>et al.</i> , 1996a,b, 2004; Friebe <i>et al.</i> , 1995c
<i>Ae. uniaristata</i>	2x	N				Badaeva <i>et al.</i> , 1996a,b; Friebe <i>et al.</i> , 1996a
<i>Ae. vavilovii</i>	6x	X ^{va} S ^{va} S ^{va}				Badaeva <i>et al.</i> , 2002
<i>Ae. ventricosa</i>	4x	N ^v D ^v				Badaeva <i>et al.</i> , 2002
<i>T. aestivum</i> deletion lines	6x	ABD	416 (deletion)			Endo and Gill, 1996; Gill and Kimber, 1974b; Gill <i>et al.</i> , 1991a

<i>T. timopheevii</i> subsp. <i>timopheevii</i>	4x	A ^t G			6	Badaeva <i>et al.</i> , 1995; Brown-Guedira <i>et al.</i> , 1996a
<i>T. timopheevii</i> subsp. <i>dicoccoides</i>	4x	AB				Gill and Chen, 1987
<i>T. monococcum</i> subsp. <i>monococcum</i>	2x	A ^m	6 (trisomics)			Friebe <i>et al.</i> , 1990b
<i>Haynaldia villosa</i>	2x	V	10			Lukaszewski, unpublished; Qi <i>et al.</i> , 1999
<i>Secale cereale</i>	2x	R	7	11		Gill and Kimber, 1974a; Mukai <i>et al.</i> , 1992
<i>S. cereale</i> deletion lines	2x	R	33 (deletion)			Friebe <i>et al.</i> , 2000a
<i>Agropyron</i> <i>intermedium</i>	6x	E ₁ E ₂ X	6			Friebe <i>et al.</i> , 1992b
<i>Elymus ciliaris</i>	4x	S ^c Y ^c	11	1		Jiang <i>et al.</i> , 1993a; Wang <i>et al.</i> , 1999
<i>E. trachycaulus</i>	4x	S ^t H ^t	7	11		Jiang <i>et al.</i> , 1993a; Morris <i>et al.</i> , 1990
<i>E. tsukushiense</i>	6x	S ^{ts} H ^{ts} Y ^{ts}	3	1	1	Wang <i>et al.</i> , 1999
<i>Hordeum chilense</i>	2x	H ^{ch}	5	1		Cabrera <i>et al.</i> , 1995
<i>Leymus racemosus</i>	4x	JN	7		2	Qi <i>et al.</i> , 1997

For chromosome addition lines, a B indicates a B chromosome addition line; for *Ae. sharonensis*, 10 different accessions were used for producing addition lines of one chromosome.

wheat species. They analyzed chromosome numbers and meiosis in wheat species and hybrids, and were the first to establish the basic chromosome number of seven and document polyploidy (2x, 4x, 6x) in the wheat group. The chromosome pairing data established that 2x and 4x wheats had one genome (AA) in common, and 4x and 6x wheat had two genomes (AABB) in common. These were exciting observations and established polyploidy as a major macrospeciation process and wheat as a great polyploidy genetic model. This method of delineating species evolutionary relationships based on chromosome pairing affinities in interspecific hybrids came to be called the genome-analyzer method (Kihara, 1954). These hybrids, of course, also could be exploited in plant breeding for interspecific gene transfers and numerous species hybrids have since been produced (Cox, 1998; Friebe *et al.*, 1996b; Jiang *et al.*, 1994a; Sharma and Gill, 1983).

Armed with the genome analyzer method, the hunt was on for the B-genome donor of 4x and 6x wheats and the extra genome (termed D-genome donor) of 6x wheat. In the 1940s, *Ae. tauschii* (syn. *Ae. squarrosa*) was simultaneously discovered in Japan and the United States as the donor of the D genome of hexaploid wheat (Kihara, 1944; McFadden and Sears, 1944, 1946). McFadden and Sears (1944, 1946) reported artificial synthesis of bread wheat by crossing tetraploid wheat with *Ae. tauschii* and chromosome doubling of the F₁ hybrid by colchicine (often the F₁ hybrids are self-fertile due to the functioning of restitution gametes). The so-called synthetic wheat, upon crossing with bread wheat, showed 21 bivalents at meiosis indicating complete chromosome homology and produced fully fertile progeny. Presumably, one or a few gametes of primitive tetraploid wheat and *Ae. tauschii* were sampled in the origin of 6x wheat from rare hybridization event that occurred in some farmer's field (as no wild 6x wheats are known in the Middle East) in the west Caspian region of Iran about 7000 years ago. Therefore, bread wheat has a very narrow genetic base, and the wheat crop was often decimated by many diseases, especially rusts. Unfortunately, the particular accession of *Ae. tauschii* used to produce the synthetic wheat was susceptible to rust (Sears, personal communication) and, hence, the notion that *Ae. tauschii* contributed little of value to bread wheat (discussed in more detail in Gill, 1993). It would take another 50 years for the full exploitation of synthetic wheats for wheat breeding (see later section).

Kihara and his colleagues undertook extensive collections of *Ae. tauschii* from its area of geographical distribution and documented extensive genetic diversity in natural populations of *Ae. tauschii* including rust resistance (reviewed in Kihara *et al.*, 1965). Kihara and coworkers also produced a large number of synthetic wheats but that remained of academic interest. In North America, Kerber and Dyck (1969), and Joppa *et al.* (1980) transferred rust and greenbug resistance to wheat from *Ae. tauschii*.

As briefly mentioned earlier, in the 1980s the WGRC launched a large-scale, sustained, and systematic effort on documenting genetic variation in *Ae. tauschii* and its rapid transfer to bread wheat by direct hybridization. We began with a small collection of *Ae. tauschii* maintained at UC–Riverside (Waines) based on the original collections of Vavilov (St. Petersburg, Russian Federation). In 1983, on the eve of the 7th International Wheat Genetics Symposium in Kyoto, Japan, Ernie Sears obtained Kihara's collection of *Ae. tauschii* for the WGRC. Our current collection of *Ae. tauschii* stands at 556 (24 duplicate) accessions. Eighteen of the 47 improved hard red winter wheat germplasm releases from the WGRC trace their pedigree to *Ae. tauschii* (Table III provides details of genes transferred).

In 1986, we began a collaborative project with CIMMYT (with Drs Byrd Curtis and Mujeeb Kazi) for the production of synthetic wheats derived from high-yielding durum and 216 accessions of *Ae. tauschii* that were shipped to CIMMYT that year. Another 40 accessions were shipped later. The synthetic wheats have played a huge role in broadening the gene pool of bread wheat. According to Maarten van Ginkel (personal communication) "by the year 2003–2004, 26% of all new advanced lines made available through CIMMYT screening nurseries to cooperators for either irrigated or semi-arid conditions were synthetic derivatives."

Another sample of 313 accessions was sent to Australia where Rudi Appels and Evans Lagudah began a large-scale program to exploit *Ae. tauschii* for wheat improvement program in that country.

Serendipitously, *Ae. tauschii* has proved to be a genetic workhorse for molecular genetic analysis of wheat and provided a window on the composition of a basic Triticeae genome (Li *et al.*, 2004). In the late 1980s, we began wheat genome mapping using restriction fragment length polymorphism (RFLP) markers and discovered that it was impractical due to the low level of polymorphism among wheat cultivars and, instead, observed a high level of polymorphism (>80% using four restriction enzymes) in a sample of *Ae. tauschii* accessions (Kam-Morgan *et al.*, 1989). Gill, *et al.* (1991) constructed the first genetic linkage map of *Ae. tauschii* and the current map consists of 730 loci incorporating placement of 160 defense-related genes (Boyko *et al.*, 2002). A high rate of recombination is the hallmark of this wild mapping population of 56 F₂ plants, where cosegregating markers have rarely been observed. In a pioneering paper, Lubbers *et al.* (1991) used RFLP markers to analyze the structure of the gene pool and define centers of genetic diversity in *Ae. tauschii* as a guide for its exploitation in wheat-improvement programs. There also were first reports of RFLP-linked markers to pest-resistance genes (Gill *et al.*, 1991; Ma *et al.*, 1993) and quantitative trait loci (QTLs) and insights into patterns of genetic introgression in wheat/*Ae. tauschii* populations (Fritz *et al.*, 1995a,b). Incidentally, the *Pst*I library genomic clone KSUD14, reported to be linked to a rust resistance gene at the distal end of 1DS arm (Gill *et al.*, 1991),

Table III
Germplasm Releases from the WGRC, Salient Traits, and Genetic Basis of Traits were Known

Germplasm	NPGS accession number	Pedigree	Resistance(s) and other traits	Gene(s)	Chromosome location(s) and/or linked markers	Reference
KS85WGRC01	PI499691	TA1644 (<i>Aegilops tauschii</i>)/Newton// Wichita	Hessian fly, soilborne mosaic virus	<i>H22</i>	1DL	Gill <i>et al.</i> , 1986a; Raupp <i>et al.</i> , 1993
KS86WGRC02	PI504517	TA1649 (<i>Ae. tauschii</i>)/ 2*Wichita	Leaf rust	<i>Lr39</i>	2DSGWM210	Raupp <i>et al.</i> , 2001; Singh <i>et al.</i> , 2003
KS87UP9	PI535771	Random-mated population	Segregating for male sterility	<i>Ms3</i>	5AWG341	Cox <i>et al.</i> , 1991b; Qi and Gill, 2001
KS89WGRC03	PI535766	TA1642 (<i>Ae. tauschii</i>)/ 2*Wichita	Hessian fly	<i>H23</i>	6DSKSUH4	Gill <i>et al.</i> , 1991d; Ma <i>et al.</i> , 1993; Raupp <i>et al.</i> , 1993
KS89WGRC04	PI535767	TA1695 (<i>Ae. tauschii</i>)/ 3*Wichita	Hessian fly, greenbug, soilborne mosaic virus	<i>Gbx</i>	7DLGDM150WMC157	Gill <i>et al.</i> , 1991c; Zhu and Smith, unpublished
KS89WGRC06	PI535796	TA2452 (<i>Ae. tauschii</i>)/TA1642 (<i>Ae. tauschii</i>)/ 2*Wichita/3/ Newton	Hessian fly	<i>H24</i>	3DLBCD451	Gill <i>et al.</i> , 1991d; Ma <i>et al.</i> , 1993; Raupp <i>et al.</i> , 1993
KS89WGRC07	PI535770	Wichita//TA1649 (<i>Ae. tauschii</i>)/ 2*Wichita	Leaf rust	<i>Lr40(Lr21)</i>	1DS (gene cloned)	Gill <i>et al.</i> , 1991b; Huang and Gill, 2001; Huang <i>et al.</i> , 2003

KS89WGRC08	PI549276	ND7532/Chaupon (<i>Secale cereale</i>)/ 4*ND7532	Hessian fly resistance; cell-culture-derived; germplasm named “Hamlet” (2B or not 2B)	<i>H21</i>	T2BS-2RL	Friebe <i>et al.</i> , 1990a; Sears <i>et al.</i> , 1992a
KS89WGRC09	PI536992	Cell-culture derived line of ND7532	Stress tolerance; from <i>in vitro</i> selection for resistance to abscisic acid	—	—	Sears <i>et al.</i> , 1992b
KS90WGRC10	PI549278	TAM107*3/TA2460 (<i>Ae. tauschii</i>)	Leaf rust	<i>Lr41</i> (may be allelic to <i>Lr39</i>)	2DSGDM35	Cox <i>et al.</i> , 1992b; Singh <i>et al.</i> , 2003
KS91WGRC11	PI566668	Century*3/TA2450 (<i>Ae. tauschii</i>)	Leaf rust	<i>Lr42</i>	1DS	Cox <i>et al.</i> , 1994b,c
KS91WGRC12	—	Century*3/TA2541 (<i>Ae. tauschii</i>)	Leaf rust (adult-plant); segregating for resistance to wheat soilborne mosaic and wheat spindle streak mosaic viruses	—	—	
KS91WGRC14	PI560335	Cando (<i>Triticum turgidum</i>)/Veery	Greenbug, leaf rust, and powdery mildew; first transfer of T1BL 1 RS to durum wheat	<i>Pm8</i> , <i>Lr26</i> , <i>Sr31</i> , <i>Yr9</i>	T1BL-1RS	Friebe <i>et al.</i> , 1993a
KS92WGRC15	PI566669	TAM200/ KS86WGRC02// Karl	Leaf rust	<i>Lr40</i>	—	Cox <i>et al.</i> , 1994c
KS92WGRC16	PI592728	Triumph 64/3/ KS8010-71/ TA2470 (<i>Ae. tauschii</i>)/TAM200	Leaf rust	<i>Lr43</i> (may be allelic to <i>Lr21</i> , <i>Lr39</i>)	7D	Brown-Guedira, unpublished; Cox <i>et al.</i> , 1997; Hussein <i>et al.</i> , 1997

(continued)

Table III (continued)

Germplasm	NPGS accession number	Pedigree	Resistance(s) and other traits	Gene(s)	Chromosome location(s) and/or linked markers	Reference
KS92WGRC17	PI592729	Vona/4/Suwon 92/ Balbo (<i>S. cereale</i>)// TAM106/3/Amigo	Hessian fly	<i>H25</i>	T6BS-6BL-6RL	Friebe <i>et al.</i> , 1991a; Mukai <i>et al.</i> , 1993; Sebesta <i>et al.</i> , 1997
KS92WGRC18	PI592730	TAM106/4/Suwon 92/Balbo// TAM106/3/Amigo	Hessian fly	<i>H25</i>	T4BS-4BL-6RL	Friebe <i>et al.</i> , 1991a; Mukai <i>et al.</i> , 1993; Sebesta <i>et al.</i> , 1997
KS92WGRC19	PI592731	Vona/4/Suwon 92/ Balbo//TAM106/ 3/Amigo	Hessian fly	<i>H25</i>	T4BS-4BL-6RL	Friebe <i>et al.</i> , 1991a; Mukai <i>et al.</i> , 1993; Sebesta <i>et al.</i> , 1997
KS92WGRC20	PI592732	TAM101/4/Suwon 92/Balbo// TAM106/3/Amigo	Hessian fly	<i>H25</i>	Ti4AS-4AL 6RL-4AL	Friebe <i>et al.</i> , 1991a; Mukai <i>et al.</i> , 1993; Delaney <i>et al.</i> , 1995a; Sebesta <i>et al.</i> , 1997
KS92WGRC21	PI566670	TAM200*3/TA2570 (<i>Ae. tauschii</i>)	Powdery mildew, wheat soilborne mosaic virus, wheat spindle streak mosaic virus	—	—	Cox <i>et al.</i> , 1994d
KS92WGRC22	PI566671	Century*3/TA2567 (<i>Ae. tauschii</i>)	Powdery mildew, wheat soilborne virus, wheat spindle streak mosaic virus	—	—	Cox <i>et al.</i> , 1994d
KS92WGRC23	PI566672	Karl*3//PI 266844/PI 355520 (<i>Triticum monococcum</i> subsp. <i>monococcum</i>)	Leaf rust	—	—	Cox <i>et al.</i> , 1994c

KS92WGRC24	PI574489	Yilmaz-4/ 2*KS84HW196	Russian wheat aphid	—	—	Martin and Harvey, 1991
KS92WGRC25	PI574490	Yilmaz-4/ KS84HW196/2/ Dodge	Russian wheat aphid	—	—	Martin and Harvey, 1991
KS93WGRC26	PI572542	Karl*3/TA2473 (<i>Ae. tauschii</i>)	Hessian fly	<i>H26</i>	4DL	Cox and Hatchett, 1994; Cox <i>et al.</i> , 1994a
KS93WGRC27	PI583794	Karl*4/CI17884	Wheat streak mosaic virus	<i>Wsm1</i>	T4DL-4Ai#2S	Friebe <i>et al.</i> , 1991b; Gill <i>et al.</i> , 1995; Wells <i>et al.</i> , 1982
KS93WGRC28	PI583795	MS6RL(6D)/ TAM104	Powdery mildew	<i>Pm20</i>	T6BS-6RL	Friebe <i>et al.</i> , 1995a
KS94WGRC29	PI986954	PI 220127//TAM200/ KS87H66	Russian wheat aphid, stem rust, leaf rust, white kernel	—	—	Martin and Harvey, 1994
KS94WGRC30	PI986955	PI 220127//TAM200/ KS87H66	Russian wheat aphid, stem rust, leaf rust	—	—	Martin and Harvey, 1994
KS94WGRC31	PI586956	PI 220350/ KS87H57// TAM200/ KS87H66/3/ KS87H325	Russian wheat aphid, stem rust, leaf rust; segregating for resistance to Hessian fly	—	—	Martin and Harvey, 1994
KS94WGRC32	PI586957	TAM107*2// KS8010-4-1/ TA359 (<i>T. monococcum</i> subsp. <i>aegilopoides</i>)	Leaf rust	—	—	

(continued)

Table III (continued)

Germplasm	NPGS accession number	Pedigree	Resistance(s) and other traits	Gene(s)	Chromosome location(s) and/or linked markers	Reference
KS95WGRC33	PI595379	KS93U69*3/TA2397 (<i>Ae. tauschii</i>)	Septoria leaf blotch, leaf rust	<i>Lr41</i>	—	
KS96WGRC34	PI604219	TAM107/TA749 (<i>T. monococcum</i> subsp. <i>aegilopoides</i>)// Wrangler	Leaf rust	—	—	Cox <i>et al.</i> , 1999b
KS96WGRC35	PI604220	Wrangler*3/TA28 (<i>Triticum</i> <i>timopheevii</i> subsp. <i>armeniicum</i>)	Leaf rust	—	—	Brown-Guedira <i>et al.</i> , 1999b
KS96WGRC36	PI604221	TAM107*3/TA870 (<i>T. timopheevii</i> subsp. <i>armeniicum</i>)	Leaf rust	<i>Lr50</i>	2BL,GWM382	Brown-Guedira <i>et al.</i> , 1999b, 2003
KS96WGRC37	PI604222	Arlin*3/TA895 (<i>T.</i> <i>timopheevii</i> subsp. <i>armeniicum</i>)	Powdery mildew; white kernel	—	—	Brown-Guedira <i>et al.</i> , 1999c
KS96WGRC38	PI604223	KS90WGRC10*3/ TA895 (<i>T.</i> <i>timopheevii</i> subsp. <i>armeniicum</i>)	Tan spot	—	—	Brown-Guedira <i>et al.</i> , 1999a
KS96WGRC39	PI604224	Wrangler*3/TA2460 (<i>Ae. tauschii</i>)	Tan spot	—	—	Brown-Guedira <i>et al.</i> , 1999a
KS96WGRC40	PI604225	KS95WGRC33 reselection	Septoria glume blotch, wheat curl mite, leaf rust	<i>Cmc3</i> , <i>Cmc4</i>	T1AL·1RS,6DS,GDM141	Cox <i>et al.</i> , 1999a; Malik <i>et al.</i> , 2003a

KS98WGRC41	—	Cando (<i>T. turgidum</i>)/ KS92WGRC20// 2* Cando	Hessian fly; first transfer of <i>H25</i> to durum wheat	<i>H25</i>	Ti4AS-4AL-6RL-4AL	Friebe <i>et al.</i> , 1999b
KS99WGRC42	—	Karl 92/PI94641(<i>T.</i> <i>turgidum</i> subsp. <i>dicoccum</i>)// 2* Jagger	Hessian fly	<i>H^{T.dic}</i>	1AS,CFA22153,BARC253	Brown-Guedira <i>et al.</i> , 2005e; Liu <i>et al.</i> , 2006
KS99WGRC43	—	Karl 92/PI94641// 2* Jagger	Hessian fly	—	1A	
KS00WGRC44	—	TAM 107*3/TA1715 (<i>Ae. tauschii</i>)	Leaf rust	—	2DS	
KS04WGRC45	—	Heyne*2//Chinese Spring*2/TA12052 (<i>Elymus</i> <i>trachycaulus</i>)	Leaf rust	—	T1H'S:1BL	Friebe <i>et al.</i> , 2005
KS04WGRC46	—	Wrangler*3/TA960 (<i>T. timopheevii</i> subsp. <i>armeniaceum</i>)	FHB	—	—	Brown-Guedira <i>et al.</i> , 2005a
KS04WGRC47	—	Karl 92*4/TA1836 (<i>Ae. speltooides</i>)	Leaf rust	—	—	Brown-Guedira <i>et al.</i> , 2005c
KS04WGRC48	—	KS94U216*2/ 92R149	Powdery mildew, leaf rust; the powdery mildew gene is from <i>Haynaldia villosa</i>	<i>Pm21,Lr21</i>	T6AL-6VS,1DS	Brown-Guedira <i>et al.</i> , 2005b
KS04WGRC49	—	Karl 92**3/TA2473 (<i>Ae. tauschii</i>)	Unique high-molecular- weight glutenin and gliadin subunits from <i>Ae. tauschii</i> ; increased loaf volume	<i>Glu-D1-1j,Glu-D1-2i</i>	1DS	Brown-Guedira <i>et al.</i> , 2005d; Knacksted, 1995

KS89WGRC5 and KS91WGRC13 were found to duplicate previously released germplasm and were withdrawn.

was sequenced as a PCR-based marker by Talbert *et al.* (1994), and proved to be the *Lr21* gene cloned 12 years later by Huang *et al.* (2003).

The *Ae. tauschii* genome, at 4000 Mb, is smaller than the A and B genomes, and essentially collinear to the D genome of bread wheat. The D genome consists of 92% repetitive DNA and 8% low-copy DNA of which 3% may be genes (Li *et al.*, 2004). However, genes are organized in clusters (Gill *et al.*, 1996a,b), and the sequencing of one such cluster revealed a 46-kb retroelement-free gene island containing seven coding sequences (Brooks *et al.*, 2002). Li *et al.* (2004) have shown that gene-rich regions can be filtered from the repetitive DNA using several approaches, especially the cot based cloning and sequencing (CBCS) method (Peterson *et al.*, 2002). A BAC-contig map of *Ae. tauschii* anchored to the genetic map is under construction (<http://wheat.pw.usda.gov/PhysicalMapping/>).

As mentioned above, the leaf rust-resistance gene *Lr21* introgressed from *Ae. tauschii* into wheat was among the first wheat genes to be isolated by map-based cloning (Huang *et al.*, 2003). Because most *Ae. tauschii*-introgressed agronomic genes lie in cotransferred, polymorphic chromosomal segments of high-gene density and recombination (Boyko *et al.*, 2002; Qi *et al.*, 2004), and most have been tagged with molecular markers, the above-mentioned genomic resources will greatly facilitate molecular cloning of these genes and open novel avenues for wheat crop improvement.

Whereas the D genome of *Ae. tauschii* and the D genome of 6x wheat recombine freely, such is not the case with transfers from A- and B-genome diploid donors to the AB genome of polyploid wheats, which share ca. 0.5 million years of coevolutionary history (Huang *et al.*, 2002). The 4A chromosome in polyploid wheats is highly rearranged and no longer pairs with 4A of diploid wheats (Chen and Gill, 1983; Naranjo *et al.*, 1987, 1988). The B-genome diploid donor has not been identified with certainty, and *Ae. speltoides* is the closest living relative (Huang *et al.*, 2002). As a result, fewer genetic transfers have been attempted from A- and B-genome diploid donors and far fewer have been agronomically desirable.

The A-genome, diploid wheat *T. monococcum* was one of the first crops to be domesticated (subsp. *monococcum*) from its wild form (subsp. *aegilopoides*) (Heun *et al.*, 1997) and is still cultivated in isolated areas. With a genome size of 5500 Mb, resources such as molecular genetic linkage map and large insert library are available (Dubcovsky *et al.*, 1996; Lijavetsky *et al.*, 1999). Because of diploidy and ease of cultivation, it is particularly suitable for mutagenesis. Vernalization genes *VRN1* and *VRN2* were recently cloned in diploid wheat (Yan *et al.*, 2003, 2004). Another sibling, A-genome, wild diploid wheat species *T. urartu* actually is now recognized as the A-genome donor of polyploid wheats (Dvorak *et al.*, 1993). The diploid wheats, both cultivated and wild forms, have good resistance to most of the common diseases of higher ploidy wheats (Gill *et al.*, 1993). New genes for leaf rust resistance were introgressed from diploid

wheat by direct hybridization in WGRC germplasm lines WGRC23, WGRC32, and WGRC34 (Table III). There is variation for crossability of different diploid wheats with common wheat. *Triticum urartu* is a poor pollen producer, and direct hybrids with common wheat have been difficult to produce. The F₁ hybrids between 6x wheat and subsp. *aegilopoides* were female fertile, and WGRC32 and WGRC34 were developed from these crosses. However, most wheat/subsp. *monococcum* hybrids were female sterile, except those with accession PI355520. The hybrid-fertility gene in PI355520 is controlled by a single dominant gene. The WGRC23 was developed by crossing the leaf rust-resistant donor accession PI266844 with PI355520, and then crossing the F₁ hybrid to a recipient wheat cultivar. Even then, there is poor pairing between putative homologous A-genome chromosomes and even some homologous pairing occurs in some hybrids (Cox *et al.*, 1991a). Upon cytological examination, WGRC23 was found to contain 40 complete chromosomes and one pair each of 6BL telocentrics and 6BS acrocentrics (Cox *et al.*, 1994c).

The putative B-genome donor, *Aegilops speltoides*, also has excellent resistance to most common diseases of wheat (Gill *et al.*, 1985). However, the degree of difficulty of genetic transfers from *Ae. speltoides* to wheat is an order of magnitude higher than from the A-genome diploids discussed above. Although hybrids are easier to produce than those involving A- or D-genome diploids, such hybrids suffer from poor homologous pairing, enhanced homoeologous pairing, and chromosome breakage. Quite a few genes for resistance to leaf rust (*Lr28*, *Lr35*, *Lr36*, and *Lr47*), stem rust (*Sr32* and *Sr39*), and one each for powdery mildew (*Pm12*) and greenbug (*Gb5*) have been transferred from *Ae. speltoides* into wheat, but none of them have as yet made any impact in agriculture (Table IV). The T7S–7A translocation involving chromosome 7S of *Ae. speltoides* and 7A of wheat in CI17884 with genes *Gb5/Lr47* was identified by Friebe *et al.* (1991b, 1996b), and further recombinants with genes *Gb5* and *Lr47* were isolated by Dubcovsky *et al.* (1998, see Table III). We have released only one line, WGRC47, containing a new, as yet unnamed, leaf rust-resistance gene extracted from *Ae. speltoides* (Table III).

Genetic transfers from diploid donors to 4x and 6x wheats probably happened rarely in nature (except the hybridization event that produced 6x wheat as discussed earlier), because the F₁ hybrid seed is normally highly shriveled and embryo rescue is routinely employed in experimental introgression research. However, hybrids between 4x and 6x wheats produce plump seed and are partially fertile. Fully fertile 4x or 6x derivatives are easily recovered upon backcrossing 5x hybrids to either parent (4x or 6x). Such enrichment of the A and B genomes of diverse land races of 6x wheats undoubtedly occurred during the 6000–7000 years of cultivation, as these genomes are relatively more polymorphic, whereas the D genome remained monomorphic (see Cox, 1998, for more details). The gene *Sr2*, which has

Table IV
Alien Transfers Derived From *Triticum* and *Aegilops* Species

Alien species	Germplasm	Alien target gene(s)	Description	Size of alien translocation	Size of missing segment	FL of break point	Mode of transfer ^a	Type ^b	Agricultural contribution ^c	Reference
<i>T. timopheevii</i> subsp. <i>timopheevii</i>	C747	<i>Sr36/Pm6</i>	T2B/2G#1				HR	C	++	Allard and Shands, 1954; Friebe <i>et al.</i> , 1996b; Jorgensen and Jensen, 1973; McIntosh and Gyrafas, 1971; McIntosh and Luig, 1973; Nyquist, 1957, 1962
	Line W	<i>Sr37</i>	T4B/4G#1				HR	C	–	Friebe <i>et al.</i> , 1996b; Gyrafas, 1968; McIntosh, 1991; McIntosh and Luig, 1973
	Thatcher/ <i>Lr18</i>	<i>Lr18</i>	T5BS-5BL-5G#1L				HR	C	–	Friebe <i>et al.</i> , 1996b; McIntosh, 1983; Yamamori, 1994
	146-155-T	<i>Pm27</i>	T6BS-6G#1S-6G#1L-6BL				MNU	C	–	Järve <i>et al.</i> , 2000
	RL6087	<i>Sr40</i>	T2BL/2G#2S				HR	C	–	Dyck, 1992; Friebe <i>et al.</i> , 1996b
<i>Ae. comosa</i>	Compair		T2DS-2M#1L-2M#1S			0.84	HR	N	–	McIntosh <i>et al.</i> , 1982; Nasuda <i>et al.</i> , 1998; Riley <i>et al.</i> , 1968a,b
	2A-2M#4/2	<i>Yr8/Sr34</i>	T2AS-2M#1L-2M#1S			0.84	HR	N	–	
	2D-2M#3/8		T2DS-2M#1L-2M#1S			0.84	HR	C	–	
	R1A, R1B, R4A, R6A	<i>Pm13</i>	T3BL-3BS-3S ¹ #1S	20–27% of 3S ¹ #1S			HR	C	–	Biagetti <i>et al.</i> , 1998; Ceni <i>et al.</i> , 1999; Ceoloni <i>et al.</i> , 1988, 1992, 1996; Donini <i>et al.</i> , 1995
<i>Ae. longissima</i>	R1D, R2A, R2B		T3DL-3DS-3S ¹ #1S	27% of 3S ¹ #1S			HR	C	–	
<i>Ae. speltoides</i>	2A/2M#4/2	<i>Lr28</i>	T4AS-4AL-7S#2S				HR	C	–	Friebe <i>et al.</i> , 1996b; McIntosh <i>et al.</i> , 1982; Naik <i>et al.</i> , 1998
	2D/2M#3/8		T4AS-4AL-7S#2S				HR	C	–	

	C95.24	<i>Sr32</i>	T2AL-2S#1L-2S#1S				HR	C	—	Friebe <i>et al.</i> , 1996b; McIntosh, 1991
	C82.1		T2BL/2S#1S				HR	C	—	
	C82.2		T2DL-2S#1L-2S#1S				HR	C	—	
	RL5711	<i>Lr35/Sr39</i>	T2B/2S#2				HR	C	—	Friebe <i>et al.</i> , 1996b; Kerber and Dyck, 1990; Seyfarth <i>et al.</i> , 1999
		<i>Pm12</i>	T6BS-6S#1S-6S#1L				HR	C	—	Jia <i>et al.</i> , 1996; Miller <i>et al.</i> , 1987
	2-9-2	<i>Lr36</i>	T6S#2S-6BS-6BL				HR	C	—	Dvorak, 1977; Dvorak and Knott, 1990
	CI17884	<i>Gb5/ Lr47</i>	T7AS-7S#1S-7S#1L	8.54 µm	0.63 µm of 7AS (size of wheat segment present)	0.85	I	C	—	Dubcovsky <i>et al.</i> , 1998; Friebe <i>et al.</i> , 1991b, 1996b; Tyler <i>et al.</i> , 1987; Wells <i>et al.</i> , 1973, 1982
		<i>Gb5</i>	Ti7AS-7AL-7S#1L-7AL	40–50 cM			HR	C	—	Dubcovsky <i>et al.</i> , 1998
		<i>Lr47</i>	Ti7AS-7S#1S-7AS-7AL	20–30 cM			HR	C	—	Dubcovsky <i>et al.</i> , 1998
	Transfer (T47)	<i>Lr9</i>	T6BS-6BL-6U#1L	0.41 µm	0.51 µm of 6BL	0.92	I	C	+	Autrique <i>et al.</i> , 1995; Friebe <i>et al.</i> , 1995c; Schachermayer <i>et al.</i> , 1994; Sears, 1956, 1972; Zhang <i>et al.</i> , 1998
<i>Ae. umbellulata</i>	T40		T6BL-6BS-6U#1L	4.65 µm	3.29 µm of 6BS	0.23	I	N	—	
	T41		T4BL-4BS-6U#1L	5.08 µm	2.90 µm of 4BS	0.23	I	N	—	
	T44		T2DS-2DL-6U#1L	1.66 µm	0.19 µm of 2DL	0.71	I	N	—	
	T52		T7BL-7BS-6U#1L	2.84 µm	1.13 µm of 7BS	0.48	I	N	—	
<i>Ae. ventricosa</i>	Roazon	<i>Pch1</i>	T7DS-7D ^v #1L				HR	C	+	Doussinault <i>et al.</i> , 1983; Jahier <i>et al.</i> , 1979, 1989, 1996
	VPM1	<i>Lr37/ Sr38/ Yr17</i>	T2AL-2AS-2M ^v #1				HR	C	+	Bariana and McIntosh, 1993, 1994; Bonhomme <i>et al.</i> , 1995; Helguera <i>et al.</i> , 2003; Seah <i>et al.</i> , 2001
	H-93-33	<i>H27</i>	DS4D(4M ^v)	N/A		N/A	N/A	N/A	—	Delibes <i>et al.</i> , 1997

^aMode of transfer is I, irradiation; or HR, homologous recombination.

^bType includes C, compensating; N, noncompensating; TC, tissue culture; S, spontaneous; EMS, EMS-induced; or NMS, *N*-methyl-*N*-nitrosourea-induced translocations.

^cAgricultural contribution listed as ++, significant; +, some; and —, none; N/A, not applicable; and FL, fraction length.

provided durable resistance to stem rust for the last 50 years, was transferred to 6x wheat from a 4x wheat land race, “Yaroslav emmer,” in the 1930s (McFadden, 1930). A QTL for high protein has been transferred to 6x wheat from wild 4x *T. turgidum* subsp. *dicoccoides* (Khan *et al.*, 2000).

As a part of a US–AID project in Morocco in the 1980s, we transferred Hessian fly resistance gene *H25* from WGRC20 (6x) to develop WGRC41 (4x) for durum wheat breeding in Morocco where Hessian fly infestations are endemic (Friebe *et al.*, 1999b). We also transferred the wheat–rye translocation chromosome T1BL·1RS, which carries a battery of resistance genes (*Pm8*, *Lr26*, *Sr31*, and *Yr9*) deployed in the world’s highest yielding wheats (6x) to 4x wheat released as WGRC14, for use in durum wheat breeding (Friebe *et al.*, 1993a). A land race accession of 4x wheat (*T. turgidum* subsp. *dicoccum*) proved to be highly resistant to US biotypes of Hessian fly, and one of the genes was transferred to 6x wheat to develop WGRC42 (Brown-Guedira *et al.*, 2005e). Molecular mapping revealed that this gene is located in a gene-rich region of chromosome 1A short arm (1AS) and closely linked with flanking markers GWM33 and CFA2153 (Liu *et al.*, 2006). The same markers also were linked with Hessian fly-resistance genes, which were, until now, erroneously mapped on chromosome 5A (Liu *et al.*, 2005b). It appears that 1AS is a hotspot of Hessian fly-resistance genes and merits further molecular analysis.

Another 4x wheat, *T. timopheevii* is a sibling species of *T. turgidum*. Its subspecies *timopheevii* is a minor crop in Transcaucasia, especially Georgia, and the wild subsp. *armeniicum* is distributed in Transcaucasia with a center of genetic diversity in northeast Iraq (Badaeva *et al.*, 1994). Pridham (1939) and Shands (1941) recognized the high level of disease resistance in *T. timopheevii* (see also Brown-Guedira *et al.*, 1996b) and attempted direct introgression of alien genes into wheat in the 1930s and transferred gene complex of *Sr36/Pm6*, which had a major impact in production agriculture (Table IV). Other transfers from *T. timopheevii* include genes for resistance to rusts and powdery mildew (*Lr18*, *Sr37*, *Sr40*, and *Pm27*). We have transferred new genes for resistance to leaf rust, powdery mildew, tan spot, and Fusarium head blight (FHB) from subsp. *armeniicum* to 6x wheat in the WGRC lines 35–38 and WGRC46. The novel resistance to FHB is noteworthy as it is the most devastating disease of wheat crop in recent years and caused over \$1.3 billion crop loss in 1993 (McMullen *et al.*, 1997).

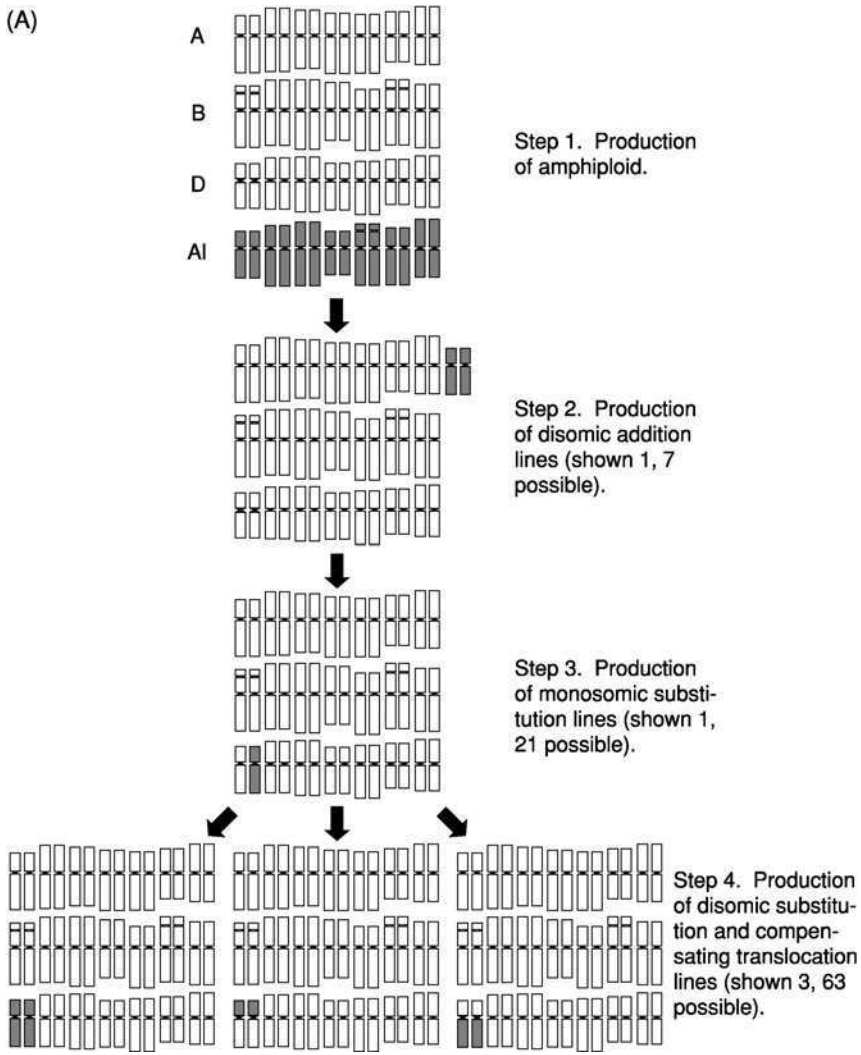
B. INTERGENOMIC TRANSFERS BY CHROMOSOME ENGINEERING

Whereas genes from genome-donor species into wheat can be transferred by homologous recombination, special techniques, such as irradiation (Sears, 1956) or induced homologous recombination (Riley *et al.*, 1968a,b),

are required for intergenomic transfers, as an example, from the R genome of rye to the A or B genomes of wheat. A flow diagram of such manipulation is presented in Fig. 3A and B. All Triticeae taxa have a basic chromosome number of $1n = 1x = 7$. Speciation in the Triticeae seems to have proceeded in two steps. First, there is a reproductive isolation by virtue of hybrid sterility or ecological preference even though the genomes are still relatively undifferentiated and capable of meiotic pairing and recombination. As an example, A-genome species hybrids between *T. monococcum* and *T. urartu* have seven ring bivalents at MI of meiosis but are sterile. Interspecific hybrids between *Ae. sharonensis* and *Ae. longissima* form 5II and 1IV (the genomes are differentiated by one reciprocal translocation) and are partially fertile. Over longer evolutionary periods of time, genomes become highly differentiated and are no longer capable of pairing, often designated by assigning different alphabetic symbols to their genomes. The genome differentiation may be nonstructural, as is the case between wheat and barley, where almost complete gene synteny and chromosome-level homology is maintained even after 12 millions of coevolution (Li and Gill, 2002). Alternatively, the genome differentiation may be structural as is the case between rye and wheat [they diverged from each other more recently, that is, 6 million years ago (Huang *et al.*, 2002)], and most rye chromosomes are highly rearranged compared to wheat and barley (Devos *et al.*, 1993). The information on the mode of genome differentiation is necessary for the choice of strategy to be used for intergenomic transfers. The method of choice for intergenomic transfer for highly rearranged alien chromosomes is irradiation and it is induced homologous pairing for syntenic alien chromosomes.

In intergenomic transfers, the production of amphiploids between wheat and alien species is the first step, followed by the isolation of alien addition, substitution, and translocation lines (Fig. 3A). Although the production of an amphiploid is highly desirable, certain combinations are resistant to doubling. In these cases, the F_1 hybrid can be directly backcrossed to produce alien addition lines. Cytological techniques, such as C-banding and genomic *in situ* hybridization and molecular marker analysis, are critical for the monitoring of alien introgression (for reviews, see Friebe *et al.*, 1996b; Jiang and Gill, 1994b; Jiang *et al.*, 1994a) as spontaneous translocations and other more complex chromosomal translocations are often encountered in backcross derivatives (Jiang and Gill, 1993; Jiang *et al.*, 1993a, 1994c). Two papers are particularly noteworthy (Friebe *et al.*, 1991b; Mukai *et al.*, 1993) as the first applications of modern chromosome analysis to complex germplasm that eventually led to the release of germplasm lines WGRC17–20 and WGRC27 (Table III). Overall, 11 of the 49 WGRC germplasm lines trace their origin to intergenomic transfers from rye (R genome), *H. villosa* (V genome), *Agropyron intermedium* (Host) Beauvois (E and X genomes), and *Elymus trachycaulus* (Link) Gould ex Shinnery (S and H genomes).

Actually, 8 of the 11 intergenomic transfers are of rye origin. As discussed earlier, WGRC14 and WGRC41 represent redeployment of rye genes from 6x to 4x wheat. WGRC8 contains the Robertsonian translocation chromosome T2BS·2RL with the 2RL of rye carrying Hessian fly resistance gene *H21* (Table III). This germplasm is late flowering and attempts have been made to reduce the size of the rye segment by homologous recombination (Ferraahi, 2001). WGRC17–20 trace their origin to breeding material



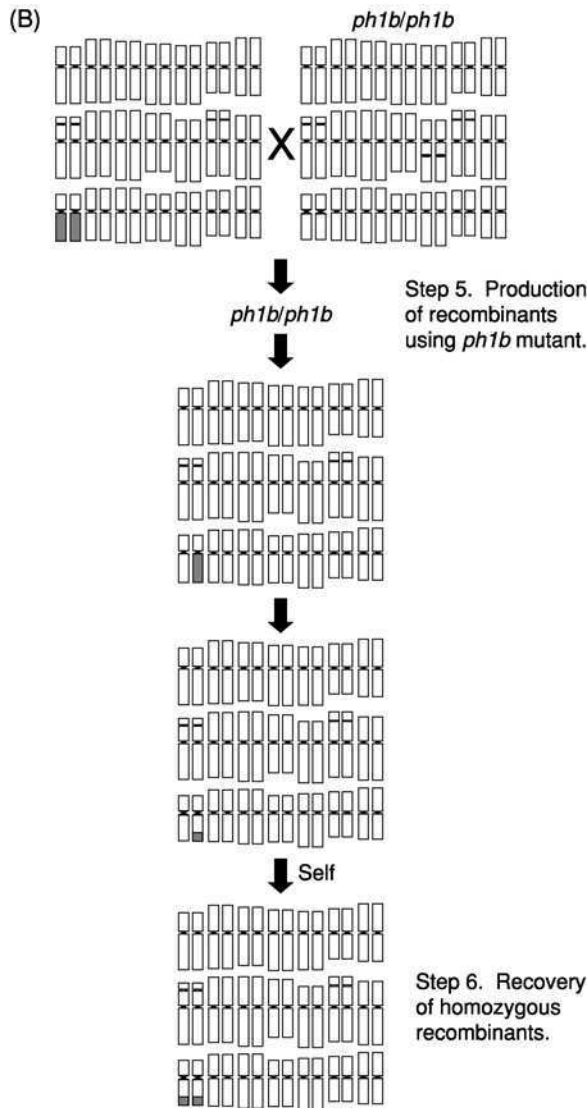


Figure 3 (A) Genetic scheme for intergenomic transfers from alien species into wheat. Production of disomic substitution and compensating translocation lines involves producing an amphiploid containing wheat (ABD) and alien (A1) genomes (Step 1), followed by production of alien chromosome disomic addition lines (Step 2), monosomic substitution lines (Step 3), and the production of disomic alien chromosome substitution or Robertsonian translocation lines (Step 4). (B) Robertsonian compensating translocation lines are the starting material for the production of wheat–alien chromosome recombinant lines by using *ph1* gene-induced homologous recombination.

developed by Emil Sebesta of USDA-ARS, Oklahoma State University. He irradiated a 6x wheat line with a pair of added 6RL telocentric chromosomes of rye carrying Hessian fly resistant gene *H25*. In retrospect, this was a good strategy, as we know now that 6R is a rearranged chromosome and contains segments derived from homologous chromosomes 6, 3, and 7 (Devos *et al.*, 1993). Sebesta, and his collaborator J. Hatchett, subjected the irradiated progenies to further breeding and agronomic selection under field conditions to isolate a number of Hessian fly-resistant lines. We analyzed these lines by molecular cytogenetic analysis to identify three different wheat-rye translocations (Friebe *et al.*, 1991a; Mukai *et al.*, 1993). One line (deployed in 6x WGRC20 and 4x WGRC41) contained a tiny rye segment inserted into wheat chromosome 4A, the first documented case of an intercalary alien transfer (Friebe *et al.*, 1991a). Postdoctoral fellow Donna Delaney identified a group-7 specific molecular marker tightly linked to *H25* at the tip of 6R that is orthologous to group 7 of the Triticeae (Delaney *et al.*, 1995a). *H25* is located in a high recombination region at the distal end and should be amenable to molecular cloning.

The development of a germplasm containing *Pm20* (WGRC28) is an example of the use of a homologous recombination between two wheat-rye addition lines derived from different rye accessions for gene transfer (Friebe *et al.*, 1994a). The original germplasm had the T6BS-6RL wheat-rye translocation chromosome carrying a fertility-restoration gene specific to *T. timopheevii* cytoplasm on 6RL. The recombinant T6BS-6RL chromosome present in WGRC28 now carries both genes. This is proof of the concept experiment of a proposal (see Friebe *et al.*, 1994a) where each basic alien Triticeae genome (seven chromosomes) should be incorporated into wheat in the form of 14 different, compensating, wheat-alien translocation chromosomes. These stocks in turn can be used as probes to extract additional genes from the donor gene pool by homologous recombination. In this way, we can cytogenetically access all the basic genomes and the vast Triticeae gene pool for wheat improvement.

We have selected *H. villosa* because its genome is already introduced into wheat as seven wheat-alien chromosome addition lines (Lukaszewski, unpublished) as the first candidate taxa for genome manipulation as proposed above. One (short arm of 6V called 6VS) of its 14 arms is already incorporated into wheat in the form of a wheat-*H. villosa* translocation chromosome T6AL-6VS and carries genes for powdery mildew and wheat curl mite resistance (Qi *et al.*, 1996). This translocation has been transferred into hard red wheat germplasm WGRC48 (Table III). For producing additional translocations, Jamie Wilson (M.S. student) crossed wheat monosomic 4D (20'' + 4D') with DA4V (21'' + 4V''), selected double monosomic F₁ plants (20'' + 4D' + 4V'), and allowed them to self. The univalent chromosomes at meiosis are prone to misdivision at the

centromeres and frequently form Robertsonian translocation chromosomes (Friebe *et al.*, 2005). In a sample of 200 plants, we identified two Robertsonian translocations for both arms and another translocation with a noncentromeric breakpoint (J. J. Wilson, unpublished). A similar strategy will be used to produce additional Robertsonian translocations for the remaining arms. These materials will be released as germplasm for extensive evaluation by the breeding community for a variety of stress resistance, physiological, quality, and agronomic traits. Those germplasm lines where *H. villosa* chromatin-controlled traits are identified will be candidates for further genomic manipulation by induced homologous recombination.

Resistance to devastating virus diseases, such as wheat streak mosaic virus (WSMV) and barley yellow dwarf virus (BYDV), is among a few traits that to a large extent are lacking in wheat. The perennial Triticeae grasses, such as *Agropyron* (in the old sense), have excellent resistance to both diseases, and breeders have been working with these sources of resistance since the 1940s. Wells at South Dakota State University developed wheat germplasm resistant to WSMV from wheat/*A. intermedium* hybrid derivatives using high pairing *Ae. speltoides* (Wells *et al.*, 1982). We analyzed this germplasm using molecular cytogenetic techniques (Friebe *et al.*, 1991b) and have identified one line containing a compensating translocation T4DL-4Ai#2S where the short arm of chromosome 4Ai of *A. intermedium* with resistance to WSMV (designated *Wsm1*) was translocated to the long arm of chromosome 4D of wheat. Obviously, this line arose from a breakage-fusion mechanism involved in the origin of Robertsonian translocations and not through recombination. It also contained an almost complete chromosome 7S from *Ae. speltoides* substituting for chromosome 7A of wheat and specified resistance to greenbug (*Gb5*). This chromosome was fixed in wheat because of its meiotic drive and, eventually, we were able to develop the WSMV-resistant line WGRC27 containing T4DL-4Ai#2S but lacking 7S (Table III). WGRC27 has been extensively used in wheat breeding, but no wheat cultivars have been released due to a yield penalty. We are now actively pursuing chromosome engineering to reduce the size of this alien segment through homologous recombination. We analyzed another WSMV-resistant germplasm line derived from wheat/*A. elongatum* derivatives, but this material was more complex and not suitable for improved germplasm development (Jiang *et al.*, 1993b). We also have been developing alien addition and translocation lines from wheat/*E. ciliaris* (SY) (Jiang *et al.*, 1993a) and wheat/*E. trachycaulus* derivatives (Jiang *et al.*, 1994c; Morris *et al.*, 1990), but no resistant germplasm to any of the viruses was developed except the recent release of a rust-resistant line WGRC45 carrying the T1BL-1H'S translocation chromosome (Table III).

Besides developing improved germplasm, we have carried out cytogenetic analysis of intergenomic transfers from many sources with a view to more

clearly define the germplasm, the mechanism of its origin, and promote further manipulation in those cases where such transfers are agronomically undesirable (Tables IV–VI). Cytogenetic analysis was used to determine if the translocations occurred between homoeologous chromosomes (called compensating translocation) or nonhomoeologous chromosomes (called non-compensating), and the compensations indices calculated based on the size of the exchanged wheat and alien segments replaced. Other aspects of these alien transfers have been discussed in detail elsewhere (Friebe *et al.*, 1996b; Jiang *et al.*, 1994a).

V. DOCUMENTATION OF GENETIC NOVELTY

Before any new gene in a germplasm for potential release can be designated and entered in the wheat gene catalog, its genetic novelty must be established by a number of criteria including recording of specific infection type to standard races of the pathogen or the insect, genetic allelism studies, and its map position on a chromosome or a genetic linkage map. A single criterion, such as a unique infection type, is not sufficient because it may be influenced by genetic background. In fact, in cases where a number of accessions of donor germplasm are resistant to all known races of the pathogen or pest, genetic analysis may be the only choice to establish the novelty of a gene in each resistant accession before resources are invested in its genetic transfer to a crop plant. In their first report, Hatchett and Gill (1981) found 5 out of 20 accessions of *Ae. tauschii* were resistant to Hessian fly biotype D, the most virulent biotype available at that time. Three were from Iran and two were of unknown origin. Further genetic studies and inheritance of resistance among resistant/resistant crosses and crosses with *H13*, the only known *Ae. tauschii*-derived source of resistance in bread wheat, showed that resistance in each accession was controlled by a single dominant gene that was different from all others (Hatchett and Gill, 1983). This documented tremendous genetic diversity for resistance to Hessian fly in *Ae. tauschii* and several of these new genes were transferred to bread wheat (Cox and Hatchett, 1994; Gill and Raupp, 1987) to develop germplasms WGRC1, WGRC3, WGRC4, WGRC6, and WGRC26 (Table III). Next, monosomic mapping was used to determine the chromosomal location of *H13* on 6D, the first gene transferred from *Ae. tauschii* to wheat (Gill *et al.*, 1987), followed by designation and monosomic mapping of other Hessian fly-resistance genes in WGRC1 (*H22* on 1D), WGRC3 (*H23* on 6D; genetic analysis was used to show that this gene is different from *H13* also located on 6D), and WGRC6 (*H24* on 3D). Later, Cox and Hatchett (1994) mapped an additional gene, *H26*, on chromosome 4D

Table V
Alien Transfers Derived from *Haynaldia villosa* and *Secale cereale* (for Description of Abbreviations, see Footnote to Table IV)

Alien species	Germplasm	Alien target gene(s)	Description	Size of alien translocation	Size of missing segment	FL of break point	Mode of transfer ^a	Type ^b	Agricultural contribution ^c	Reference
<i>H. villosa</i>	KS04WGRC48	<i>Pm21/Cmc</i>	T6AL·6V#1S	6VS	6AS	0	1	C	+	Chen <i>et al.</i> , 1995, 1996; Liu <i>et al.</i> , 1999; Qi <i>et al.</i> , 1996
<i>S. cereale</i>	<i>T. aestivum</i> cultivars Aurora and Kavkaz	<i>Pm8/Sr31/Lr26/Yr9</i>	T1BL·1R#1S	1RS	1BS	0	S	C	++	Bartos and Bares, 1971; Bartos <i>et al.</i> , 1973; Friebe <i>et al.</i> , 1989, 1996b; Lukaszewski, 1993; Mettin <i>et al.</i> , 1973; Ren <i>et al.</i> , 1997; Rogowski <i>et al.</i> , 1993; Schlegel and Korzun, 1997; Zeller, 1973; Zeller <i>et al.</i> , 1982
	<i>T. durum</i> KS91WGRC14 MA1, MA2	<i>Pm8/Sr31/Lr26/Yr9/Gli-B1/Glu-B3</i> (lacking <i>Sec-1</i>)	Ti1R#1S _{40;9} ; 44:38·1BL Ti1R#1S _{40;9} ; 44:45·1BL	1RS ^{rec}	1BL	0	HR	C	—	Friebe <i>et al.</i> , 1987, 1989, 1993a Lukaszewski, 2000

(continued)

Table V (continued)

Alien species	Germplasm	Alien target gene(s)	Description	Size of alien translocation	Size of missing segment	FL of break point	Mode of transfer ^a	Type ^b	Agricultural contribution ^c	Reference
	Amigo	<i>Gb2/Pm17</i> (allelic to <i>Pm8</i>)	T1AL·1R#2S	1RS	1AS	0	I	C	++	Heun <i>et al.</i> , 1990; Hollenhorst and Joppa, 1981; Hsam and Zeller, 1997; Hsam <i>et al.</i> , 1995; Jiang <i>et al.</i> , 1994b; Lowry <i>et al.</i> , 1984; Lukaszewski, 1993; Sebesta and Wood, 1978; Sebesta <i>et al.</i> , 1995b; The <i>et al.</i> , 1992; Zeller and Fuchs, 1983
	GRS 1201	<i>Gb6</i>	T1AL·1R#3S	1RS	1AS	0	I	C	—	Porter <i>et al.</i> , 1991, 1994
	GRS 1204	<i>Gb6</i>	T2AL·2AS·1R#3S T2AS·1R#3S·1RL#3L			0.39 in S 0.27 in L	I	N	—	Friebe <i>et al.</i> , 1995e
	Transec	<i>Dn</i> <i>Lr25/Pm7</i>	T1BL·1R#4S T4BS·4BL·2R#1L	1RS 2.40 µm	1BS 1.03 µm of 4BL	0 0.61	HR I	C N	— —	Marais <i>et al.</i> , 1994 Driscoll and Anderson, 1967; Driscoll and Bielig, 1968; Driscoll and Jensen, 1963, 1964, 1965; Friebe <i>et al.</i> , 1996b; Heun and Friebe, 1990

ST-1	<i>Lr45</i>	T2AS-2R#3S- 2R#3L	1.71 µm	1.58 µm	0.39	I	C	—	McIntosh <i>et al.</i> , 1995a; Mukade <i>et al.</i> , 1970
WRT238		T3AS-3R#1S	3RS	3AL	0	I	N	—	Acosta, 1962; Friebe <i>et al.</i> , 1996b
90M126-2	<i>Sr27</i>	T3AL-3R#1S	3RS	3AS	0	I	C	—	Friebe <i>et al.</i> , 1996b; Marais and Marais, 1994
90M129-9	<i>Pm20, rf</i>	T3BL-3R#1S	3RS	3BS	0	I	C	—	Friebe <i>et al.</i> , 1994a, 1995a; Heun and Friebe, 1990; Porter and Tuleen, 1972
KS93WGRC28		T6BS-6R#3L	6RL	6BL	0	S	N	—	
KS85HF011 KS89WGRC8 Hamlet	<i>H21</i>	T2BS-2R#2L	2RL	2BL	0	TC	C	—	Friebe <i>et al.</i> , 1990a, 1999b; Lee <i>et al.</i> , 1996; Sears <i>et al.</i> , 1992a; Seo <i>et al.</i> , 1997
88HF16KS92	<i>H25</i>	T6BS-6BL-6R#1L	6.95 µm		0.11	I	N	—	Delaney <i>et al.</i> , 1995a; Friebe
WGRC17									<i>et al.</i> , 1991a,
WGRC18		T4BS-4BL-6R#1L	3.88 µm		0.40	I	N	—	1999b; Mukai
WGRC19		T4BS-4BL-6R#1L	3.88 µm		0.40	I	N	—	<i>et al.</i> , 1993;
WGRC20		Ti4BS-4AL-6R#1L- 4AL	0.70 µm		0.06, 0.19	I	N	—	Sebesta <i>et al.</i> , 1997

Table VI
Alien Transfers Derived from *Agropyron* Species (for Description of Abbreviations, see Footnote to Table IV)

Alien species	Germplasm	Alien target gene(s)	Description	Size of alien translocation	Size of missing segment	FL of break point	Mode of transfer ^a	Type ^b	Agricultural contribution ^c	Reference
<i>A. elongatum</i> (<i>Thinopyrum ponticum</i> 2n = 10x = 70)	Agatha	<i>Lr19/Sr25</i>	T7DS·7DL-7Ac#1L	2.55 µm	2.62 µm of 7DL	0.32	I	C	+	Dvorak and Knott, 1977; Friebe <i>et al.</i> , 1994b; Knott, 1968; McIntosh <i>et al.</i> , 1977; Sharma and Knott, 1966
	Agatha-28	<i>Lr19/Sr25</i>	T7DS·7DL-7Ac#1L	2.73 µm	2.71 µm of 7DL	0.29	EMS	C	—	Friebe <i>et al.</i> , 1994b; Knott, 1980
	Agatha-235	<i>Lr19</i>	Ti7DS·7DL-7Ac#1L·7DL	1.99 µm	1.29 µm of 7DL	0.31, 0.75	EMS	C	—	Friebe <i>et al.</i> , 1994b; Knott, 1980
	7Ag#11	<i>Lr29</i>	T7DL·7Ac#1L·7Ac#1S				HR	C	—	Friebe <i>et al.</i> , 1996b; McIntosh <i>et al.</i> , 1995b; Procnier <i>et al.</i> , 1995; Sears, 1973, 1978
	Agent	<i>Sr24/Lr24</i>	T3DS·3DL-3Ac#1L	1.26 µm	1.38 µm of 3DL	0.70	S	C	++	Dedryver <i>et al.</i> , 1996; Friebe <i>et al.</i> , 1996b; Jiang <i>et al.</i> , 1994a; McIntosh <i>et al.</i> , 1977; Schachermayr <i>et al.</i> , 1995; Smith <i>et al.</i> , 1968

	Teewon	<i>Sr24/Lr24</i>	T1BL·1BS-3Ae#1L			0.50 in the satellite of 1BS	I	N	—	Friebe <i>et al.</i> , 1996b; Jiang <i>et al.</i> , 1994b; Sebesta <i>et al.</i> , 1995a; The <i>et al.</i> , 1992
	K2046	<i>Sr26</i>	T6AS·6AL-6Ae#1L	2.48 µm	3.63 µm of 6AL	0.09	I	C	++	Dundas and Shepherd, 1998; Friebe <i>et al.</i> , 1994b; Knott, 1961, 1968
	CI15322	<i>Wsm</i>	T4DS·4DL-1Ae#1L	1.31 µm	0.73 µm of 4DL	0.67	I	N	—	Friebe <i>et al.</i> , 1996b; Jiang <i>et al.</i> , 1993b; Martin <i>et al.</i> , 1976; Pfannenstiel and Niblett, 1978; Sebesta and Bellingham, 1963; Sebesta <i>et al.</i> , 1972
	875-94-2	<i>Cmc2</i>	T5BL·6Ae#2S	6Ae#2S	5BS	0	S	C	—	Friebe <i>et al.</i> , 1996b; Kim <i>et al.</i> , 1992; Whelan and Hart, 1988; Whelan <i>et al.</i> , 1983
<i>A. inter- medium</i>	KS93WGRC27	<i>Wsm1</i>	T4DL·4Ai#2S	4Ai#2S	4DS	0	I	C	—	Friebe <i>et al.</i> , 1991b; Gill <i>et al.</i> , 1995; Talbert <i>et al.</i> , 1996; Wells <i>et al.</i> , 1973, 1982
	CI17883	<i>Wsm1</i>	T6AL·4Ai#2S T6AS·4Ai#2L	4Ai#2S 4Ai#2L	6AS 6AL	0 0	I	N	—	Friebe <i>et al.</i> , 1991b; Wells <i>et al.</i> , 1973, 1982

(continued)

Table VI (continued)

Alien species	Germplasm	Alien target gene(s)	Description	Size of alien translocation	Size of missing segment	FL of break point	Mode of transfer ^a	Type ^b	Agricultural contribution ^c	Reference
	A29-13-3	<i>Wsm1</i>	T4AL-4Ai#2S	4Ai#2S	4AS	0	HR	N	—	Chen <i>et al.</i> , 1998; Liang <i>et al.</i> , 1979; Wang and Liang, 1977; Wang and Zhang, 1996; Wang <i>et al.</i> , 1977; Wells <i>et al.</i> , 1982
	T4	<i>Lr38</i>	T3DL-3DS-7Ai#2L	2.78 µm	0.67 µm of 3DS	0.46	I	N	—	Friebe <i>et al.</i> , 1992d, 1993b; Wienhues, 1960, 1966, 1967, 1971, 1973, 1979
	T7	<i>Lr38</i>	T6DS-6DL-7Ai#2L	4.19 µm	1.45 µm of 6DL	0.32	I	N	—	
	T24	<i>Lr38</i>	T5AL-5AS-7Ai#2L	4.20 µm	0.88 µm of 5AS	0.35	I	N	—	
	T25	<i>Lr38</i>	T1DS-1DL-7Ai#2L	2.55 µm	0.82 µm of 1DL	0.59	I	N	—	
	T33	<i>Lr38</i>	T2AS-2AL-7Ai#2L	2.42 µm	1.40 µm of 2AL	0.62	I	N	—	

	86–187	<i>Sr44</i>	T7DS-7Ai#1L·7Ai#1S		HR	N	—	Cauderon, 1966; Cauderon <i>et al.</i> , 1973; Friebe <i>et al.</i> , 1996b; McIntosh, 1991
	TC6	<i>Bdv</i>	T7DS-7Ai#1S·7Ai#1L	0.33	TC	C	—	Banks <i>et al.</i> , 1995; Cauderon, 1966; Cauderon <i>et al.</i> , 1973; Friebe <i>et al.</i> , 1996b; Hohmann <i>et al.</i> , 1996
	TC7	<i>Bdv</i>	T1BS-7Ai#1S·7Ai#1L	0.37	TC	N	—	
	TC14	<i>Bdv</i>	T7DS·7DL-7Ai#1L	0.56	TC	C	—	Ayala <i>et al.</i> , 2001; Banks <i>et al.</i> , 1995; Cauderon, 1966; Cauderon <i>et al.</i> , 1973; Friebe <i>et al.</i> , 1996b; Hohmann <i>et al.</i> , 1996
<i>Th. distichum</i>	Indis	<i>Lr19/Sr25</i>	T7DS·7DL-7Ae#1L		S	C	+	Friebe <i>et al.</i> , 1996b; Marais and Marais, 1990; Marias <i>et al.</i> , 1988; Prins <i>et al.</i> , 1996

in WGRC26. Ma *et al.* (1993) identified molecular markers linked to both genes and confirmed chromosome mapping results for *H23* and *H24*. Liu *et al.* (2005a) have identified a microsatellite marker cosegregating with *H13* at the distal end of 6D short arm (6DS), a result contrary to the previous reported location on 6D long arm based on telosomic mapping (Gill *et al.*, 1987). A reexamination of the pedigree results showed that a wrong telosomic stock was used in the arm mapping experiment. These data also call for reevaluation of the relationship between *H13* and *H23* for which molecular marker data now show that both are located in the distal region of 6DS. Liu *et al.* (2006) have identified a molecular marker at the tip of chromosome 1AS cosegregating with a new Hessian fly gene transferred from dicoccum in WGRC42. The same marker also is tightly linked with genes *H9*, *H10*, and *H11*, indicating that they all map on chromosome 1A and not on 5A as reported previously (Liu *et al.*, 2005b).

Unlike Hessian fly, genetic analysis of leaf rust resistance in *Ae. tauschii* showed widespread occurrence of *Lr21* alleles in Iran (Miller, 1991). The molecular analysis showed that the *Lr40* gene in WGRC7 (derived from *Ae. tauschii* accession TA1649, collected in Iran) was allelic to *Lr21* (derived from TA1599, also collected in Iran, see Rowland and Kerber, 1974), and molecular cloning has confirmed this (Huang and Gill, 2001; Huang *et al.*, 2003). A mistake was discovered in the released WGRC2 line, as it was identical to WGRC7 (Huang and Gill, 2001). Since then, original seed of WGRC2 has been evaluated, and it contains *Lr39* derived from TA1675 and is located on 2DS (Raupp *et al.*, 2001). In addition, it has been discovered that *Lr41* in WGRC10 previously located on 1D by monosomic mapping (Cox *et al.*, 1994b) is allelic to *Lr39* in 2DS arm (Singh *et al.*, 2003). WGRC16 was reported to have a gene designated *Lr43* located on 7D by monosomic mapping (Hussein *et al.*, 1997). Segregation analysis and evaluation with markers for *Lr21* and *Lr39* indicated that in fact, WGRC16 carries the gene combination *Lr21* and *Lr39* (Brown-Guedira, unpublished data). These analyses indicate that *Lr39* also may be widespread in *Ae. tauschii* since this gene appears to have been transferred from multiple accessions. The *T. turgidum* subsp. *armeniacum*-derived gene *Lr50* in wheat germplasm WGRC36 was mapped to 2B long arm and is the first leaf rust-resistance gene located on that chromosome arm (Brown-Guedira *et al.*, 2003). *Lr50* was also transferred to wheat from several different accessions of *T. turgidum* subsp. *armeniacum*. The molecular mapping is ongoing for all the remaining leaf rust-resistant WGRC lines and a clearer picture of diversity of leaf rust-resistance genes should emerge in the near future.

In molecular analysis of other germplasm, dominant male sterility gene *Ms3* (in KS87UP9) has been tagged with molecular markers located

in the proximal region of 5AS (Qi and Gill, 2001). Wheat curl mite resistance gene *Cmc4* in WGRC40 has been located in 6DS and tagged with a molecular marker (Malik *et al.*, 2003a). Genes on alien segments transferred by intergenomic manipulation have been characterized only as to the identity of chromosome segments involved as analyzed by C-banding and *in situ* hybridization (see Tables IV–VI), but their molecular analysis is more problematic. How this kind of analysis must be undertaken is illustrated by molecular mapping of *H25* transferred from rye and tagged with a molecular marker located 1.7 cM from the gene (Delaney *et al.*, 1995a).

VI. GERMPLASM FOR WHEAT-BREEDING PROGRAMS

A primary goal of the WGRC, from its earliest days, has been to develop new germplasm from interspecific and intergeneric crosses and release it in a genetic background that will encourage its use by public and private wheat breeders. The WGRC has made germplasm available in two ways: (i) through formal release by the Kansas State University Agricultural Experiment Station, cooperating experiment stations, and/or the USDA–ARS and (ii) through submission of entries in the USDA–ARS Regional Germplasm Observation Nursery (RGON).

From 1985 through 2004, the WGRC issued 48 germplasm releases (Table III). Most of these lines were registered in the journal *Crop Science* and deposited with the National Plant Germplasm System. Release notices were sent to research and breeding organizations in the United States and around the world.

In all, but three of the germplasm lines, the primary traits were resistances to pathogens, insects, or mites. Nine carried chromosomal translocations involving alien segments; most of the remainders were derived from hybridization with *Aegilops* and *Triticum* species, followed by homologous recombination. Because the WGRC's intention is to expand the gene pool of wheat with useful genetic diversity not previously available, much effort has been focused on determining the genetic basis of the traits expressed by these germplasm lines. Allelism studies, monosomic analysis, linked markers, molecular cytogenetics, and other methods have provided information on the locations of genes in most of the released lines (Tables IV–VI).

The WGRC has not only concentrated on problems of economic importance in the US hard winter wheat region (e.g., leaf rust, Hessian fly, virus diseases, wheat curl mite, Septoria leaf and glume blotch, tan spot, Russian wheat aphid, and heat stress) but also has released germplasm

that addressed problems of greater relevance in other regions (e.g., powdery mildew and *Fusarium* head blight). For recurrent parents, researchers generally used hard winter wheat cultivars or experimental lines adapted to the central and southern Great Plains. However, two of the releases were durum wheats with unique chromosomal segments from rye (Table III).

The second route of germplasm dissemination has been through the RGON, to which breeders and geneticists throughout the hard winter wheat region submit early-generation lines for evaluation and observation. Lines are evaluated for at least eight traits, with testing for each trait done by cooperators at one or more appropriate sites in the region. The RGON is coordinated by the USDA-ARS Wheat, Sorghum, and Forage Unit at Lincoln, Nebraska, which distributes the data to all interested members of the wheat research community. WGRC scientists entered approximately 80 lines in the RGON from 1996 to 2004 and distributed seed in response to any subsequent requests.

For germplasm, one indicator of relevance is its frequency in pedigrees of advanced lines and cultivars. Breeders in the central and southern US hard winter wheat region enter some of their most advanced lines in the Southern Regional Performance Nursery (SRPN). The 2005 SRPN, sown in 2004, had 44 experimental entries. The numbers of entries that have had WGRC germplasm lines or RGON entries as direct parents (i.e., in the final cross before selection) have risen steadily from one or two in 1996–1998 to nine in 2005 (Fig. 4). Those lines were derived from WGRC parents distributed in the late 1980s and throughout the 1990s (Fig. 4).

Of course, germplasm has a practical impact on agriculture only when it is used to develop cultivars. The lag time between release of a germplasm line and the release of a cultivar descended from that line is longer than the lag time for breeding lines that was evident in Fig. 4. By 2004, WGRC parents had appeared in the pedigrees of three hard winter wheat cultivars, “Overley,” Agripro “Thunderbolt,” and Agripro “Fannin,” and the soft red winter wheat “Rachael.” WGRC10 is one of the parents of the Croatian cultivar “Talija.” WGRC parents have been used frequently by the wheat-breeding program at CIMMYT (van Ginkel, M., personal communication).

VII. THE NEXT 25 YEARS

It is worth projecting what the WGRC will look like in the next 25 years. The WGRC was a dream that became a reality and had tremendous growth during the last 25 years, far outpacing the infrastructure, staff needs, and the

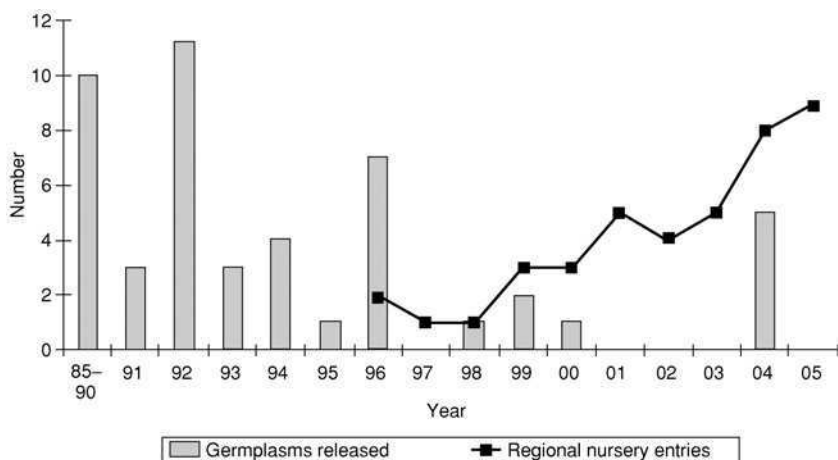


Figure 4 Numbers of germplasm releases by the WGRC from 1985 through 2004 (bars) and numbers of entries in the Southern and Northern Regional Performance Nurseries from 1996 through 2005 in which at least one immediate parent was developed by the WGRC.

operating budget. Yet the challenge for the next 25 years to the year 2030 is even greater. Population growth and economic development are putting a huge stress on the natural range of the wild wheat species and relatives that are the wellspring of genetic diversity, the capital for crop improvement programs. The concept that we could sample the natural genetic diversity and conserve it in our gene banks is only partially true. We know now that stress resistance genes against environmental assault are fast evolving and for these we must have sufficiently large populations over diverse geographic regions so that such evolutionary processes can go on in nature in response to the ever changing and man-made environmental conditions. Thus, we must pay attention to *in situ* conservation. For rational decisions on the areas to be conserved and collections to be made, we must understand the structure and distribution of genetic diversity of our crop plant relatives. We must survey what we have in our gene banks, after eliminating duplicated collections, and analyze collections from areas of genetic diversity for further collections and *in situ* conservation. For harnessing the natural genetic variation for crop improvement, we must have extensive and intensive knowledge of crop plant morphology, physiology, and especially the genomic knowledge at the level of chromosomes structure and behavior, and down to the DNA sequence. This will require a huge investment in genetic and genomic tools, resources, and infrastructure. To reflect this and to meet this challenge, we have redefined the mission of the WGRC to the

Wheat Genetics and Genomic Resources Center (WGGRC). The WGGRC will strive its best to serve the wheat genetics community in this new environment. The WGGRC will promote and conduct collaborative research on the following objectives:

1. Collect, maintain, evaluate, document, and conserve regions of high and useful genetic diversity and distribute wheat genetic and molecular resources.
2. Develop and distribute improved germplasm using traditional and novel genetic technologies for enhancing and sustaining crop production and productivity.
3. Develop and distribute genetic stocks especially to facilitate functional analysis of the wheat genome.
4. Conduct wheat genome mapping and sequencing of genes and allele mining to facilitate marker-assisted breeding and value-added trait development.
5. Conduct training and outreach.

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CULTIVATION OF *STEVIA* [*STEVIA REBAUDIANA* (BERT.) BERTONI]: A COMPREHENSIVE REVIEW

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Acknowledgments
References

Stevia rebaudiana (Bert.) Bertoni is one of the 154 members of the genus *Stevia*. It is a sweet herb of Paraguay. The leaves of the shrub contain specific glycosides, which produce a sweet taste but have no caloric value. For centuries, this herbal sweetener has been used by native Guarani Indians to counteract the bitter taste of various plant-based medicines and beverages. Many countries have shown interest in its cultivation, and research activities have been initiated. Incorporation of this species in agricultural production systems, however, depends upon a thorough knowledge of the plant and its agronomic potential. The published literature on research and development of this crop is meager. The aim of this chapter is to describe the ecology, importance of the plant, and its production requirements, but major emphasis is given to the agronomic and management aspects of the plant to be grown as a crop. Further, this chapter represents an effort to compile the literature on *S. rebaudiana* and review the current status of understanding of the plant and its potential as an alternate source of cane sugar.

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I. INTRODUCTION

Stevia rebaudiana (Bert.) Bertoni is one of the 154 members of the genus *Stevia*. It is a sweet herb of Paraguay, which contains natural noncaloric sweetener. It is of immense value due to its adaptability to wide climatic range, the high-sweet content, and its significant contribution to the welfare of human life. This offers a solution for complex diabetic problems and obesity in humans, being calorie free. The worldwide demand for high potency sweeteners, particularly natural sweeteners, is expected to increase in the years to come. This assumes center stage in the society, which is under the organic and natural food regime. Incorporation of this species in agricultural production systems, however, depends upon a thorough knowledge of the plant and its agronomic potential. The published literature on research and development of this crop is meager. The review of the literature in past emphasized on nutritional qualities and safety of stevioside, rather than *Stevia* cultivation (Felippe, 1977, 1978; Fletcher, 1955). Further, a review from China was recently done by Liu (1992).

The aim of this chapter is to describe the ecology and importance of the plant and its production requirements, but major emphasis is given to the agronomic and management aspects of the plant to be grown as a crop. Further, this chapter represents an effort to compile the literature on

S. rebaudiana and review the current status of understanding of the plant and its potential as an alternate source of cane sugar.

II. AGRICULTURAL HISTORY

Stevia rebaudiana is an endemic herb from Paraguay and the Brazilian border with that country (Felippe, 1977; Monteiro, 1982). The genus *Stevia* (Eupatorieae, Asteraceae) [which consists of approximately 150–200 species of herbaceous, shrub and subshrub plants (Gentry, 1996)] is one of the most distinctive genera within the tribe Eupatorieae. Schmeling (1967) expressed that *Stevia* is mainly found in Amambay, including the zone of San Pedro, Yhu, and near Jejui Guazu. It was used for many centuries as a sweetener. *Stevia* first came to the attention of Europeans in the 1800s, yet it remained relatively obscure until it was planted and used in England during the sugar rationing of World War II. Many authors described the herb (Klages, 1951; Levy, 1984). History of *Stevia* was dealt in detail by Machado and Dietrich (1981).

Although geographically widespread, this genus occurs exclusively in tropical and subtropical regions of the United States and Central and South America (Robinson and King, 1977). This has been historically used by the people of Paraguay as a sweetener and herbal remedy. Early reports indicated that *Stevia* was known to the Spanish during the 16th century, but it remained in obscurity until it was again brought to the attention of Europeans in 1888 by M. S. Bertoni. Erstwhile *Eupatorium rebaudianum* came to the attention of M. S. Bertoni in the year 1809, who studied it and renamed it as *Stevia* in 1905 (Bertoni, 1899, 1905, 1918, 1927). Prior to any European discovery, it had long been known to the indigenous Guarani people native to that region. The leaves of this sweet herb, known to the Guarani Indians as Ka-a He-e, was used for centuries as a sweetener for bitter drinks such as maté (Soejarto *et al.*, 1983). *Stevia* is reported to be originated in South American gene center (Cerna, 2000).

During 1971, Japanese introduced *Stevia* from Brazil (Crammer and Ikan, 1986) and conducted research to evaluate potential of *Stevia*. Today, Japan is a major grower and marketer for the sweetener and has approved it for use in many food products, including cereals, teas, and soft drinks. *Stevia* has an ancient and venerable history in certain parts of the world. It is clear that this crop is native to valley of the Rio Monday in North Eastern Paraguay and is commonly found on the edges of marshland on acid infertile sand or muck soils of Paraguay. In Canada, *Stevia* is being sold as an ingredient in tea but not as a sweetener (Borie, 2000). The task now is to convert *Stevia* from its wild habitat to a modern crop well suited to different production environments along with efficient mechanized production.

III. AGRICULTURAL IMPACT AND USE

Stevia possesses numerous characteristics that make it a potentially valuable agricultural species (Tables I and II), although there are few reasons generally limiting its agronomic utility (Table III). Information on production of the 10 glycosides responsible for its sweetness in different plant parts is of great importance for both understanding the peculiarities of diterpenoid glycoside production and for adoption of mass scale production techniques. Studies conducted so far could suggest few management approaches for improving production requirements. This crop had made significant agricultural impact in countries such as Japan, China, Taiwan, Korea, Mexico, USA, Thailand, Malaysia, Indonesia, Australia, Tanzania, Canada, Abkhazia, Russia (Brandle and Rosa, 1992; Chen and Chang, 1978; Chu and Cheng, 1976; Donalisio *et al.*, 1982; Dzyuba, 1998; Goenadi, 1983; Gvasaliya *et al.*, 1990; Katayama *et al.*, 1976; Lee *et al.*, 1979; Lester, 1999; Saxena and Ming, 1988; Shock, 1982; Sumida, 1968), and efforts were initiated in India recently (Chalapathi, 1996).

Randi (1980) reviewed the potential uses of *Stevia* that produces sweet glycosides like stevioside, which may vary from 2 to 10% (Magalhaes, 2000), a noncaloric sweetener that does not ferment in the human body (Table II).

The leaves are used for sweetening, as is, or dried and pulverized, or soaked in water; the liquor is used for sweetening beverages. A Japanese firm is producing chewing gum from *Stevia*. Plants contain an aromatic resin, which has tonic action on digestive organs. It is also a source of gibberellin (Duke and deCellier, 1993). The herbage contains 0.12–0.16% essential oil, which is up to 0.43% in the inflorescence (Kingham and Soejarto, 1985).

Table I
Agronomically Important Characters of *Stevia*

Serial number	Characters
1	Wide climatic adaptability
2	Perennial in nature (Andolfi <i>et al.</i> , 2002), unique regeneration capacity after frost injury (Singh and Kaul, 2005)
3	Leaf is the economic part
4	Vegetative propagation is possible (Chalapathi <i>et al.</i> , 1997b)
5	3–4 harvests per year is possible (Donalisio <i>et al.</i> , 1982)
6	Intercropping is possible during the initial growing period
7	Easy propagation through seeds, stem cuttings, and division of roots (Singh and Kaul, 2005).

Table II
Product (Glycoside) Suitability Characters

Serial number	Characters
1	Improves cardiovascular functioning (Machado <i>et al.</i> , 1986)
2	Effective in high blood pressure, obesity or chronic yeast infections (Elkins, 1997)
3	Diabetic safe (Kingham and Soejarto, 1985; Soejarto <i>et al.</i> , 1983)
4	Antihuman rotavirus activity (Takahashi <i>et al.</i> , 2001)
5	Calorie free—human physiology cannot metabolize the sweet glycosides contained in <i>Stevia</i> leaves, therefore, they are eliminated from the body with no caloric absorption (Elkins, 1997)
6	Improved overall gastrointestinal function (Alvarez, 1986)
7	Can be used in baking because its sweet glycosides do not break down when heated (Elkins, 1997)
8	Hypoglycemic action: Positive (Oviedo <i>et al.</i> , 1970; Soejarto <i>et al.</i> , 1983); Negative (Akashi and Yokoyama, 1975; Lee <i>et al.</i> , 1979); Inconclusive (Boeckh, 1986; Piheiro and Gasparini, 1981)
9	<i>Stevia</i> leaves also contain protein, fibers, carbohydrates, phosphorus, iron, calcium, potassium, sodium, magnesium, rutin (flavonoid), zinc, vitamin C, and vitamin A (Elkins, 1997)
10	Does not adversely effect blood sugar levels (Elkins, 1997)
11	Effective against microbes like <i>Streptococcus mutans</i> , <i>Pseudomonas aeruginos</i> , and <i>Proteus vulgaris</i> (Yabu <i>et al.</i> , 1977)
12	50–400 times sweeter than white sugar (Elkins, 1997)
13	Nontoxic (Elkins, 1997)
14	Inhibits the formation of cavities and plaque (Elkins, 1997)
15	Contains no artificial ingredients (Elkins, 1997)

Table III
Agronomically Challenging Characters

Serial number	Characters
1	Day length sensitivity/short day plant (Lester, 1999; Valio and Rocha, 1966)
2	Sensitive to water logging
3	Low to moderately resistant to drought (Jia, 1984)
4	Poor early growth (Borie, 2000)
5	Heavy weed competition at early stages (Andolfi <i>et al.</i> , 2002)
6	Sensitivity to frost
7	Poor seed germination (Barathi, 2003; Carneiro <i>et al.</i> , 1997; Duke, 1993; Shock, 1982)
8	Short period of germinantive power (Marcavillaca, 1985)
9	Poor tolerance to high soil pH (Shock, 1982)
10	Self incompatible (Chalapathi <i>et al.</i> , 1997b)
11	Asynchronous seed maturity

IV. BOTANICAL DESCRIPTION

It is one of the 950 genera of the Asteraceae family (Lester, 1999; Soejarto *et al.*, 1983). A systematic study of the North and Central American species of *Stevia* was done by Grashoff (1972). Although there are more than 200 species in the *Stevia* genus, Soejarto *et al.* (1983) had proved that *S. rebaudiana* gave the sweetest essence. It is a perennial herb with an extensive root system and brittle stems producing small, elliptic leaves (Shock, 1982).

Kingdom: Angiospermae

Class: Dicotyledons

Group: Monochlamydae

Order: Asterales

Family: Asteraceae

Subfamily: Asteroideae

Tribe: Eupatorieae (Cabrera *et al.*, 1996)

Genus: *Stevia*

Species: *rebaudiana*

Stevia is normally described as perennial herb in its natural habitat in Paraguay, though under some environmental conditions and management situations it behaves as an annual or mixture of plants of both types. The cultivated plants reported to be more vigorous. It is also considered as a branched bushy shrub (Dwivedi, 1999). Since leaves are the principal sweet bearing parts of the plant, the proportion of leaf to whole plant, the leaf weight ratio is important. High ratios of leaf:stem are desirable in cultivated *Stevia* because of the low stevioside concentrations ($<5 \text{ mg g}^{-1}$) in stem tissue. *Stevia* grows to about 50–60 cm tall (Brandle and Rosa, 1992; Lester, 1999), 100 cm (Shock, 1982), or up to 120 cm (Dwivedi, 1999).

A. GROWTH PATTERN

Stevia has a temperamental nature that is often reflected in its sluggish growth when the plants are first set out. After the first month, they pick up growth depending upon the prevailing weather conditions. Branching and tillering are also much more profuse (Shock, 1982). The growth pattern of *Stevia* can be divided into four stages: germination, grand growth period, flowering, and seed maturity. The first stage includes germination and establishment, the second vegetative growth, the third floral bud initiation to pollination and fertilization, and the fourth seed growth and filling. The duration of sowing to seed emergence is related to the temperature, and 24°C is considered optimal for seed germination (Goettemoeller and Ching, 1999). Other growth stages are discussed in detail under environmental versatility.

B. PLANT MORPHOLOGICAL VARIATION

Monteiro (1980) studied the morphological differences present in these plants but was unable to separate them in to valid taxonomic varieties. There are reports of irregularity of the quantitative and qualitative production of the sweetening molecules from cultivated *S. rebaudiana* types also. The morphological differences between plants, so evident in present cultivation at Institute of Himalayan Bioresource Technology (IHBT), Palampur (Ramesh, unpublished data) as well as elsewhere in the world, are also linked to the natural reproductive biology of the species (*S. rebaudiana* belongs to the half-SIB species) (Tateo *et al.*, 1998). The diversity of the aerial part of the cultivated plants (Tateo *et al.*, 1998) as well as flowering behavior (Zaidan *et al.*, 1980) was large, and they identified three photoperiod classes based on day length, which needs further research for suitability for cropping conditions in different parts of the world.

C. ROOT SYSTEM

The root is fibrous, filiform, and perennial, forming abundant stock (Schmeling, 1967) that is hardly ramified and does not deepen, distributing itself to the land surface; and is the only part that does not contain stevioside (Vargas, 1980; Zaidan *et al.*, 1980). Sunk (as quoted by Taiariol, 2004) described that the fine roots congregate around the soil-surface and thicker roots in the deepest zones.

D. STEM

The stem is annual, subligneous, more or less pubescent, with tendency to decline, and more or less graft (Sakaguchi and Kan, 1982).

E. LEAVES

The first photosynthetic organs are formed after germination from the two cotyledons in the seed. They are rounded in shape. *Stevia* has an alternate leaf arrangement and herbaceous growth habit with flowers arranged in indeterminate heads. Leaves are small, lanceolate, oblong, serrate, and sweet (Dwivedi, 1999). For *Stevia*, the leaf area index (LAI) at 80 DAS was 4.83 (Fronza and Folegatti, 2003). Light or, more exactly, photosynthetically active radiation supplies plants with energy for photosynthate production. It is fairly obvious that the amount of intercepted light

principally depends on the leaf surface area of the crop, and is usually expressed as leaf area index.

F. FLOWERS

Stevia is self-incompatible (Chalapathi *et al.*, 1997b; Miyagawa *et al.*, 1986) and probably insect pollinated plant (Oddone, 1997). The flowers are small and white (Dwivedi, 1999) with a pale purple throat (Figs. 1, 2, 3, 4, 5, and 6). The pollen can be highly allergenic. The tiny white florets are perfect, borne in small corymbs of two to six florets. Corymbs are arranged in loose panicles (Goettemoeller and Ching, 1999). A plant takes more of a month in producing all its flowers (Taiariol, 2004).

G. SEEDS

Shock (1982), Duke (1993), and Carneiro *et al.* (1997), had reported poor percentage of viable seeds in *Stevia*. Oddone (1997) considers “clear” seeds to be infertile. Seeds are contained in slender achenes, about 3 mm in length. Each achene has about 20 persistent pappus bristles. Reproduction in the



Figure 1 Corolla of single *Stevia* flower.



Figure 2 Flower cluster of *Stevia*.

wild is mainly through seed, but seed viability is poor and highly variable (Lester, 1999). Seeds have very little endosperm and are dispersed in the wind via hairy pappus. A study undertaken to investigate the low seed germination of *Stevia* seeds through artificial pollination treatments as a means to increase seed germination revealed that some active manipulation of the blossoms is necessary to achieve pollination (Goettemoeller and Ching, 1999).

H. SWEET GLYCOSIDE CONTENT IN PLANT PARTS

There are 10 glycosides out of which stevioside and rebaudioside A are important. Details of the sweet glycosides are dealt in the Section VII. Plant organs contained different amounts of the sweet glycoside, stevioside, which declined in the following order; leaves, flowers, stems, seeds, and roots. Root was the only organ that does not contain steviosides. This made Metivier

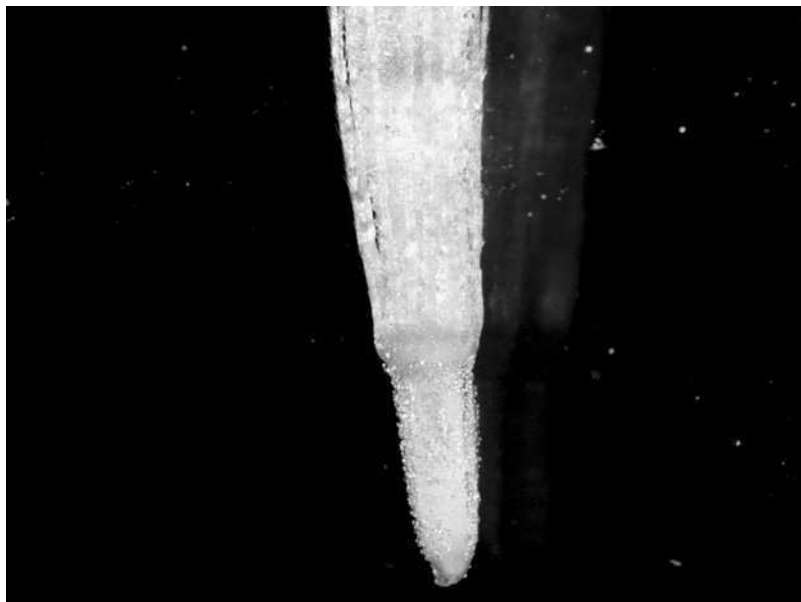


Figure 3 Single flower (Bottom view).

and Viana (1979a) to hypothesize that stevioside may protect the aerial portions of the plant from herbivore predators. The sweetness in the leaves is two times higher than that in inflorescence (Dwivedi, 1999). The highest amount of steviosides was found in the upper young actively growing shoot sections, whereas lowest senescent shoot sections exhibited the lowest amount of such compounds. During ontogeny, a gradual increase in the stevioside concentration was observed in both mature *Stevia* leaves and stems, and this process lasted up to the budding phase and the onset of flowering (Bondarev *et al.*, 2003b).

V. ENVIRONMENTAL VERSATILITY

The objective of this section is to describe and discuss briefly the relationships between selected environmental variables and the agronomic responses of *Stevia*. *Stevia* has been successfully grown apparently under variety of geographic locations around the world, although it originated in the high-land regions of northeastern Paraguay that occur between 23 and 24° S latitude (Shock, 1982), and 54 and 56° E longitude (Alvarez, 1984; Bertonha



Figure 4 Single flower (Top view).

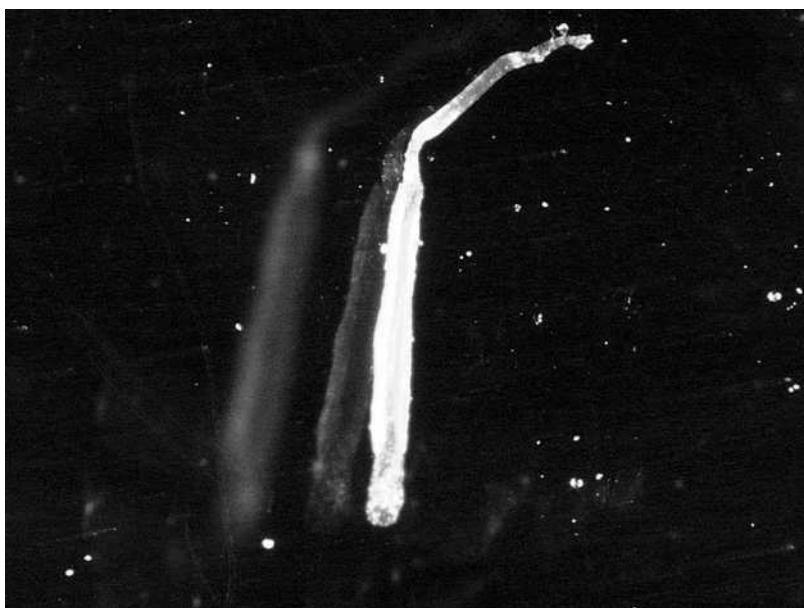


Figure 5 *Stevia* stamen.



Figure 6 *Stevia* stigma.

et al., 1984; Monteiro, 1986). It is this extreme versatility that holds importance for this plant. *Stevia* is grown as a perennial crop in subtropical regions including parts of the United States, while grown as an annual crop in mid to high latitude regions (Goettemoeller and Ching, 1999). The results indicate that agronomic yield mainly depend on the genetic characters of the plant and the phenotypic expression, which ultimately is governed by the climatic and environmental factors (Ermakov and Kotechetov, 1996; Metivier and Viana, 1979a). Moreover, synthesis of terpenes is affected by them in many plants (Guenther, 1949; Krupski and Fischer, 1950; Langston and Leopold, 1954). Selected locations where *Stevia* is grown is presented in Table IV.

In association with most plants, the growth and flowering of *Stevia* are affected by radiation, day length, temperature, soil water, and by wind in exposed places. As early in 1976, the seasonal variation in stevioside content was studied by Chen *et al.* (1978). Tateo *et al.* (1999) had opined that environmental and agronomic factors have more influence on stevioside production than the growth habit. For *Stevia* crop, the ideal climate can be considered as semi-humid subtropical with temperatures ranging from -6 to 43°C with an average of 23°C (Brandle and Rosa, 1992). Research conducted at Egypt revealed that climate conditions such as temperature, length, and intensity of photoperiod greatly affected *Stevia* production and

Table IV
Selected Locations Where *Stevia* is Grown

Location	Latitude ^a	Longitude ^a
Bangalore, India (Chalapathi <i>et al.</i> , 1997b)	12° N	77° E
Hosur, India (Barathi, 2003)	13° N	77° E
Sugar Crops Research Institute, ARC-Giza, Egypt (Allam <i>et al.</i> , 2001)	25° N	30° E
New Delhi, India	29° N	77° E
Uttaranchal, India	30° N	77° E
Palampur, India	32° N	76° E
California (Shock, 1982)	38° N	
Western Georgia (Papunidze <i>et al.</i> , 2002)	42° N	44° E
Pisa, Italy (Fronza and Folegatti, 2003)	43° N	11° E
Abkhazia (Eastern coast of black sea) (Gvasaliya <i>et al.</i> , 1990)	45° N	70° E
Suwon, Korea (Lee <i>et al.</i> , 1980)	37° 30' N	127° E
Slovakia (Cerna, 2000)	49° N	20° E
Czech republic (Nepovim <i>et al.</i> , 1998a)	50° N	15° E
Canada (Lovering and Reeleader, 1996)	50–60° N	130° W
Moscow, Russia (Kornienko <i>et al.</i> , 1995)	55° 45' N	37° 42' E
Indonesia (Goenadi, 1987)	0–5° S	110–120° E
Sukabumi, Indonesia (Basuki, 1990)	7° S	110° E
Brazil (Stefanini and Rodrigues, 1999)	25° S	50° W
Argentina (Cabanillas and Diaz, 1996)	30° S	60° W

^aApproximate and not the exact location where experiment was done.

quality as evident from the remarkable increase in yield during the summer cuts than that during winter cuts (Allam *et al.*, 2001).

Brandle and Rosa (1992) had reported comparable stevioside concentration at Delhi and Ontario to that found in Japan where long days are experienced during the growing season (Kinghorn and Soejarto, 1985) relative to the subtropical regions of the world, which might be due to cultivation under long days at Delhi and Canada. Under agro-climatic conditions at Palampur, stevioside content in leaf varied from 3.17 to 12% and from 1.54 to 3.85% in stem as estimated during the studies at IHBT, Palampur. Stevioside content in selected locations where *Stevia* is grown is presented in Table V.

A. GEOGRAPHIC DISTRIBUTION

Bertoni (1905) had described the distribution range of 22°30'–25°30' S latitudes and from 55 to 57° W longitudes, while Sunk (1975) described it more precisely as 22–24° S and 55–56° W, respectively, within 200–700 m altitudinal zones. The native habitat of *Stevia* is at latitude of 25° S in a subtropical region in northeastern Paraguay between 500 and 1500 m above

Table V
Stevioside Content in Selected Locations Where *Stevia* is Grown

Serial number	Place	Stevioside content (%)	Reference
1	San Piero a Grado, Pisa, Italy	6.49 (80 DAS)	Fronza and Folegatti (2003)
2	Academy of Sciences of Czech Republic, Flemingovonam, Prague	5.2 (July)–6.5 (September)	Nepovim <i>et al.</i> (1998a)
3	Minga Guazu region of East Paraguay	4.36–9.89	Tateo <i>et al.</i> (1999)
4	Paraguay	10.23–13.46	Tateo <i>et al.</i> (1999)
5	Argentinian origin	5.16	Pryluka and Cernadasn (1985)
6	Brazilian origin	5.4	Pryluka and Cernadas (1985)
7	Palampur, India	8–10 (Average); 3.17–12 (Extreme range)	Based on present R&D at the Institute IHBT, India
8	Place not known	6–20	Donalisio <i>et al.</i> (1982)
9	Bangalore, India	9.08	Ashwini (1996) and Chalapathi (1996)
10	Western Georgia	10	Papunidze <i>et al.</i> (2002)

msl, with an average annual temperature of 25°C and an average rainfall of about 1375 mm year⁻¹ (Shock, 1982; Sumida, 1973). Kawatani *et al.* (1977) had surveyed *Stevia* plantation in southeast Asian countries and found taller plants near the tropic, but leaf productivity was less due to higher proportion of stems than leaves in these latitudes. This made him to conclude that subtropical latitudes are favorable for higher leaf recovery. European commission (1999) has opined that this is growing mostly at altitudes of 500–3000 m in semidry mountainous terrain. However, this perennial shrub has been grown at 60° N also, weathering the bitter chill of St Petersburg winters, but essentially it prefers tropical climate between 35 and 45° to either side of the equator (Midmore and Rank, 2002).

B. DAY LENGTH/PHOTOPERIOD

Stevia is highly sensitive to the day length and it requires 12–16 h of sunlight. This prompted many investigators to examine the effect of length of day and night and temperature variation on the cultivation and the resultant stevioside levels (Kudo, 1974; Metivier and Viana, 1979a; Mizukami *et al.*, 1983; Valio and Rocha, 1966; Viana, 1981; Zaidan *et al.*, 1980).

Day length variation had a profound influence on crop vegetative growth. This was confirmed by the studies conducted by Metivier and Viana (1979a). The results revealed that plants maintained under long day conditions were characterized by long internodes and a single main stem bearing large, horizontally held ovate leaves, whereas it was rosette up to bolting and thereafter internode length increased. Besides, this influenced flowering also.

Precise investigations on day length and time required for flowering were made by Kudo (1974), describing that flowering occurred within 46 days at 11 h day length, while it was extended to 96 days when photoperiod was extended for 12.5 h. Kudo and Koga (1977) found that under optimum day length conditions flowering started from 50 to 60 days after sowing and was also confirmed by Zaidan *et al.* (1980). However, Valio and Rocha (1966) opined that a photoperiod of 13–14 h might be necessary. The plant flowered in the 8, 10, 12, and 13 h photoperiod, though the highest percentage of flowering occurred in the 13 h photoperiod. This made researchers to believe that *Stevia* is an obligate short day plant (Lester, 1999) with a critical day length of about 13 h. Since glycoside synthesis is reduced at or just before flowering, delayed flowering with long days allowed more time for glycoside accumulation. Thus, *Stevia* production is best suited to a long day environment, where vegetative growth is longer and steviol glycoside yields will be higher. This was confirmed further by Metivier and Viana (1979a). They claimed that yield of sweetening compounds present in leaf tissue varied according to day length, as long days increased leaf area and leaf dry weight as compared to short days. Therefore, enhanced vegetative growth under long day conditions is not surprising as the high leaf:stem ratio may be a function of cultivation under long days (Brandle and Rosa, 1992). Apart from increment in leaf yield, 50% increase in stevioside concentration relative to short days was also observed (Metivier and Viana, 1979a).

In its native habitat, at 21–22° S latitude *Stevia* plants start flowering from January to March equivalent to July and September in the northern hemisphere. Subsequent flowerings occur in rapid succession as regrowth from the plant crown grows shorter each time until winter in July (Shock, 1982). If *Stevia* is grown at about 25°C, under continuous long day conditions (16-h photoperiod), it will remain in vegetative stage itself (Monteiro *et al.*, 2001). Undoubtedly, short days promote flowering and so day length influences yield (Parsons, 2003) of aerial biomass.

Photoperiod requirements made researchers to conclude that cultivation in temperate areas with long summer days would be ideal for high stevioside yields but seed production would be difficult (Shock, 1982). In accordance with the above views, this is grown as a perennial crop in subtropical regions, where longer days favor leaf yield and stevioside contents (Goettemoeller and Ching, 1999). Flowering under short day conditions should occur

54–104 days following transplantation in southern hemisphere, depending on the day-length sensitivity of the cultivar (Lester, 1999).

It is worthwhile to mention that rooting of cutting is also dependent on day length, since Zubenko *et al.* (1991) had recorded better rooting and growth of cuttings made in April as compared to February due to increased day length and light intensity. If it is possible to screen a suitable genotype to suit day-length sensitivity, the plant's total potential can be fully exploited.

C. TEMPERATURE

Temperature has been observed to influence on the availability of soil nutrients, germination, the growth of the plant and shoots, winter survival, photosynthesis, and respiration. It is rather difficult to delineate a particular process that is most affected by temperature. But it is commonly believed from the early work of Sumida (1980) (and as cited by Sakaguchi and Kan, 1982) that the optimal temperature range for the growth of *Stevia* is 15–30°C, though the plant can tolerate critical temperature of 0–2°C. However, Miyasaki (and as cited by Sakaguchi and Kan, 1982) showed the absolute limit as little as –3°C. Mizukami *et al.* (1983) had postulated that day night temperature variation is another determinant for stevioside production. They obtained best growth and stevioside yield under 25/20°C day/night regime. Notwithstanding to these findings, Nepovim *et al.* (1998a) had concluded that temperature was not a decisive parameter for the stevioside content in the *Stevia* leaves, which is a controversy. Temperature influences yield (Parsons, 2003) either directly or through the diurnal variation as it plays a vital role in *Stevia* production as discussed by Barathi (2003), indicating that the maximum day temperature should not exceed 40°C and the night temperature should not fall below 10°C for favorable growth of *Stevia*.

A report made by Midmore and Rank (2002) on possibility of introducing *Stevia* in Australia indicated that vegetative growth is reduced when temperature is below 20°C.

In subtropical India, it has been successfully grown at a temperature regime of 28–39°C (Chalapathi *et al.*, 1997b). Richard (2004) had stated that the average temperatures where *Stevia* is found growing ranges from –6 to 44°C and these areas are humid.

D. LIGHT

Studies conducted by Metivier and Viana (1979b) had indicated that to keep the plants in vegetative stage, light intensity of 0.089 cal cm^{–2} should be

maintained. *Stevia* is essentially a sun loving plant since the plant thrived in a warm, humid, and sunny climate (Jia, 1984). Under natural habitat it grows wild along with tall grasses under partial shade. Hence, the productivity is poor. The negative effect of shade was further confirmed by the observations made by Slamet and Tahardi (1988). They confirmed that shade reduced growth and rate of flowering. Further, about 60% reduction in light delayed the flowering, decreased plant biomass production, significantly decreased the percentage of flowering plants, and also reduced rate of flowering. Observations at IHBT, Palampur, India also confirmed that growth was reduced under partial shade.

VI. CULTIVATION

Stevia cultivation has been reported as early as 1970s (Mitsuhashi *et al.*, 1975; Miyazaki *et al.*, 1978). In the early phase of cultivation, *Stevia* crop exhibited much more vigor than in natural populations (Shock, 1982), a fact suggesting that with appropriate crop management practices we could expect luxuriant crop with fullest potential. Nowadays commercial cultivation is extended/attempted in Japan, southeast Asia, and United States (Fors, 1995; Sakaguchi and Kan, 1982), but it is being cultivated in some semitropical areas, humid Himalayan hilly regions, and humid hills of Assam in India (Dwivedi, 1999). As the plant does not survive winter climate, it is cultivated in Europe as a leaf crop under greenhouse conditions (European Commission, 1999). There have been studies on development of modern techniques of cultivation, propagation through tissue culture, and selection (Acuna *et al.*, 1997; Akita *et al.*, 1994; Ashwini, 1996; Ferreira and Handro, 1988a,b,c; Filho *et al.*, 1992; Flachslund *et al.*, 1996; Handro *et al.*, 1993; Huang *et al.*, 1995; Kornilova and Kashnikova, 1996; Nepovim and Vanek, 1998b; Patil *et al.*, 1996; Sivaram and Mukundan, 2002; Tamura *et al.*, 1984b). Considerations regarding the cultivation practices are discussed hereunder.

A. SEED GERMINATION, NURSERY, AND CROP ESTABLISHMENT

In general, seed germination is a problem in *Stevia* (Felippe and Lucas, 1971; Randi, 1980; Randi and Felippe, 1981; Rocha and Valio, 1972). Seeds sown in cold weather showed poor germination (Shock, 1982). Alvarez *et al.* (1994) had reported that it was impossible to sow the seeds immediately after harvest, and concluded that the seeds should be kept in sealed tight



Figure 7 *Stevia* nursery through cuttings.

containers in the refrigerator at 4°C, since it loses viability at room temperature. Further, studies indicated that germination was best at 25°C (Felippe and Randi, 1984; Randi and Felippe, 1981) and at this temperature, 63.2% of maximum germination (90.03%) occurred after 101.4 h (Takahashi *et al.*, 1996). Cabanillas and Diaz (1996) had reported the performance of seeds under different temperature and light conditions at Argentina.

No viable seed treatment to enhance seed germination has been reported elsewhere. Because of its small size and the related bottlenecks in seed nutrition, it is a general practice to raise nurseries. It is propagated through either seeds or cuttings (Figs. 7 and 8). Seeds are germinated in the glasshouse in spring and the plants (usually 6–7 weeks old) are transplanted into the field (Lester, 1999). In the temperate latitudes, the production cycle for annual crops starts with the 6–7 weeks old plants grown from seed. Under Canadian conditions the initial establishment was very poor (Brandle *et al.*, 1998). The seedlings raised from seeds are transplanted and the shoot is harvested after 4–5 months of growth (Dwivedi, 1999). Seeds were stored for 11 months at 4°C or at ambient temperature and humidity (Cabanillas and Diaz, 1999).

B. SPACING/CROP DENSITY

Crop density is a parameter decided by the crop spread above ground so as not to interfere with the development of the adjoining plants. However,



Figure 8 *Stevia* nursery raised through seed.

one should consider the root spread also. This is also dependent on the environment in which it is raised. Several authors tried different spacing (Angkapradipa *et al.*, 1986b; Barathi, 2003; Basuki, 1990; Carneiro *et al.*, 1992; Chalapathi, 1996; Columbus, 1997; Donalisio *et al.*, 1982; Katayama *et al.*, 1976; Murayama *et al.*, 1980).

Initial trials indicated that higher growth and yield, when low plant density was adopted (60×20 cm), while dry leaf yield was higher in denser planting (60×10 cm) (Murayama *et al.*, 1980). In contrast, Lee *et al.* (1980) had reported that plant height, number of branches, and number of nodes were unaffected by planting density (50–70 cm between and 10–30 cm within rows), but dry leaf yield per plant decreased with increasing plant density. In accordance to the above, Donalisio *et al.* (1982) had recommended a plant population of 80,000–100,000 plants ha^{-1} .

Reduction in row-to-row spacing was also attempted. A spacing of 50×20 cm (Filho *et al.*, 1997a) or 45×22.5 cm (Chalapathi, 1996) performed well but still narrow spacing of 25×25 cm was also tried by Angkapradipa *et al.* (1986b), however, this is not advisable considering the root spread of

the crop. Observations made at IHBT, Palampur indicated that, at 12 months after planting, the root spread was 30 cm on either side suggesting that for a multiple harvest crop the spacing should be higher than 30 cm on either side.

Basuki (1990) tried a very high density of 2 lakh plants ha^{-1} to manage weeds. However, this would result in poor crop growth due to intense light competition and the leaf:stem ratio will decline. Leaf yield was found to increase up to 1.1 lakh plants ha^{-1} for the first year of production (Brandle *et al.*, 1998). Under Palampur conditions, 50,000 plants ha^{-1} were maintained at a spacing of 45×45 cm (Singh and Kaul, 2005). The highest *Stevia* yield was obtained at 70×25 cm spacing at Abkhazia (Gvasaliya *et al.*, 1990). Therefore, it is advisable to carry out trials in each planting zone to establish adequate plant population density for that particular area.

C. VEGETATIVE PROPAGATION

Propagation of *Stevia* is usually by stem cuttings, which root easily but require high labor inputs. Poor seed germination is one of the factors limiting large-scale cultivation.

1. Method of Propagation

a. Cuttings Gvasaliya *et al.* (1990) had reported that nearly 98–100% rooting was obtained, when current year's cuttings were taken from leaf axils at Abkhazia. Rooting of cutting was best (96.7%) in cuttings from side shoots and from tops of the main shoot (92.3%). Further, cuttings from the top part of the main stem with four internodes generally gave the best results (Tirtoboma, 1988). However, the pair of leaves in the cutting as well as the season also act as determinants for the rooting percentage. Cuttings with four pairs of leaves rooted poorly, especially in February. In February, cuttings with two pairs of leaves rooted best and in April those with three pairs of leaves (Zubenko *et al.*, 1991). Cuttings of 8 cm long were used by Carvalho *et al.* (1995).

Use of 15 cm cutting gave significantly higher sprouting percentage with better shoot and root growth of sprouted cuttings over 7.5 cm cuttings (Chalapathi *et al.*, 1999c, 2001), while direct planting in field was of limited success only (Chalapathi *et al.*, 1999c).

b. Rooting of Cuttings and Their Growth Pretreatment of cuttings with IBA, IAA, and its combination @ 1000 ppm caused callus injury due to higher concentration of growth regulators (Chalapathi *et al.*, 1999c), while

paclobutrazol at 50 or 100 ppm was effective in inducing roots and sprout from stem cuttings (Chalapathi *et al.*, 2001) under pot conditions. Plant growth and stevioside content in the leaves of the plants grown from stem tips were more uniform than in plants grown from seeds. Number of roots, above ground biomass and stevioside content were greater in the vegetative grown plants (Truong and Valicek, 1999).

c. Time of Planting There is scant published information on this aspect. However, the optimal time of planting is primarily decided by the avoidance of climatic conditions, which militate its stand and establishment. Summer is always associated with dry weather and poor soil moisture conditions hindering crop establishment. Further, late autumn planting is associated with poor temperature and less time for plant development. Therefore, planting at the initiation of spring seems to be the best option. Plants are more productive when seedlings or rooted cuttings are set out as early as possible in the spring (Lee *et al.*, 1979). Under northern hemisphere, planting is done during mid May (Brandle *et al.*, 1998). Under the agro-climatic conditions of Palampur the ideal time of planting was observed to be during March–April so as to have two leaf harvests and one seed harvest in a crop (Ramesh, personal communication). Further, delayed planting during June–July resulted in poor leaf harvest as it entered flowering during September in Northern hemisphere, at Palampur, India.

Winter cereal growing is an established practice in many parts of the world. Therefore, possibilities of raising this crop along with winter cereal remain to be a challenge. Under practical considerations, several other factors and local farming situations determine the time of planting. In brief, raising nursery during winter under controlled environments offers a reliable solution so that plating can be taken up in the subsequent spring.

2. Method of Propagation on Sweet Glycosides Content

This is only a matter of leaf growth rather than for examining stevioside content in plants. Tamura *et al.* (1984a) had compared plants raised from seeds, cuttings, and stem tip culture and concluded that yield of sweetening compounds present in leaf tissue can vary according to method of propagation, while Nepovim *et al.* (1998a) had contradicted the former and stressed that the content of stevioside did not depend on the type of propagation. Since the crop is cross-pollinated, there must be variation in the advancing generations, thus, obtaining varying stevioside content. Variation in stevioside content in a population of *Stevia* was reported (Tateo *et al.*, 1998). Therefore, plants developed from cuttings would be more uniform in growth with optimum stevioside concentration. This

suggested that vegetatively propagated material is the best propagule for higher stevioside productivity.

D. NUTRIENT MANAGEMENT

Nutrient requirements of this crop are low (Goenadi, 1987) to moderate since this crop is adaptable to poor quality soils in its natural habitat at Paraguay. When placed under commercial culture, for economic crops, manuring is necessary (Donalisio *et al.*, 1982; Goenadi, 1985). Since leaf is the economic part of this crop, it is presumed that higher nutrient application may aid in higher yield. But only few works have been carried, mainly on nutritional aspects.

The visual symptoms of nutrient deficiency in *Stevia* were: N exhibiting yellowing of leaves, P as dark green leaves, and chlorotic and mottled leaves with K deficiency. Further, the secondary nutrients deficiencies were exhibited viz., apical necrosis, chlorosis and inverted "V" shaped necrosis, and small pale green leaves for Ca, Mg, and S, respectively (Utumi *et al.*, 1999).

In tissue culture studies, it was found that changes in the composition of the nutrient medium may significantly modify the physiological processes (Sikach, 1998) and production of the steviol glycosides in *Stevia* tissues and exert in such a manner physiological regulation of this process (Bondarev *et al.*, 1998).

1. Macronutrients

Results from Japan demonstrated that, at the time of maximum dry matter accumulation, *Stevia* consisted of 1.4% N, 0.3% P, and 2.4% K (Katayama *et al.*, 1976). It is an established fact that nutrient application is better than no manuring and was also experimentally proved by Murayama *et al.* (1980) and Goenadi (1985), who obtained better growth rate and dry leaf yield than no manuring. This was further strengthened by Lee *et al.* (1980) who had recorded increase in leaf yield with moderate application of nitrogen, phosphorus, and potassium fertilizers in Korea.

Early studies with nitrogen nutrition by Kawatani *et al.* (1977) had indicated an increase in growth, stem thickness, and number of branches. Response to potassium was also obtained (Kawatani *et al.*, 1980). The crop would require approximately 105 kg N, 23 kg P, and 180 kg K for a moderate biomass yield of 7500 kg ha⁻¹ under Canadian conditions (Brandle *et al.*, 1998), thus suggesting the importance of fertilization. Deficiency of N, K, and Mg reduced vegetative growth in terms of leaf growth,

which ultimately reduced marketable part of the plant. However, Mg impaired root growth also to a greater extent. N, P, K, and S deficiencies decreased the shoot:root dry weight ratio, while it is reverse for Mg deficiency. Except Ca, all others decreased absorption of macronutrients (Utumi *et al.*, 1999). This study suggests that a balanced use of fertilizers is an absolute necessity.

Besides improvement in growth, research conducted at Egypt showed a gradual and significant increase in fresh and dry leaves, stem, biomass yields, and total soluble carbohydrate as nitrogen fertilizer increased from 10 to 30 kg N. Dry leaves yield increased by 64 and 1.99% at the later dose as compared to lower dose (Allam *et al.*, 2001).

In an Andosol with a pH of 4.5, N had no significant effect but P and K increased biomass production (Angkapradipta *et al.*, 1986b). Increasing rate of N increased plant N content, whereas P and K did not do so in a latosol (Angkapradipa *et al.*, 1986a).

If the nutritional requirements of the crop were established, it would suggest us the need for fertilization either through organic means or inorganic means. This was attempted by Son *et al.* (1997) at Brazil. They concluded that shortly before or at flowering the production of 1 ton of dry leaves, demanded in kg: N-64.6, P-7.6, K-56.1, Ca-15.8, Mg-3.6, and S-3.6. In accordance with these findings, in a ratoon crop at Bangalore, growth and yield increased significantly with increasing rates of N, P, and K up to 40:20:30 kg ha⁻¹ with highest dry leaf yield. In India, responses were obtained in terms of nutrient uptake (Chalapathi *et al.*, 1997a) for fertilization, growth and yield up to 60:30:45 kg NPK ha⁻¹ (Chalapathi *et al.*, 1999b) at Bangalore.

Further, the nutritional demand for seed production is still higher than leaf production, which was reported to be, in kg, N-130, P-18.8, K-131.5, Ca-43.7, Mg-8.3, and S-9.7 (Son *et al.*, 1997) for 1 ton.

2. Micronutrients

There appears to be poor requirement for the microelements. Since this crop prefers acid soils with low pH, this condition itself ensured adequate availability of micronutrients. However, even in acid soils response was noticed. The decreasing order of response of *Stevia* to microelements when sprayed in an acidic soil in terms of plant fresh weight was as follows: 0.1% Mn > 0.05% Mo > 0.02% Mo > 0.05% Zn > 0.1% B > 0.05% Mn > 0.02% Cu > 0.25% B > 0.2% Zn (Zhao, 1985). Experiments conducted in nutrient solutions indicated that Boron supplied at 10 ppm reduced growth, flowering, root weight, and caused leaf spotting also (Sheu *et al.*, 1987). Filho *et al.* (1997a) had studied the micronutritional requirements of *Stevia* at Brazil. They concluded that shortly before or at flowering the production of 1 ton of

dry leaves, demanded in g: B-89, Cu-26, Fe-638, Mn-207, and Zn-13. For seed production corresponding to 1 ton of dry leaves, the extraction of micronutrients, in g, was B-226, Cu-76, Fe-2550, Mn-457, and Zn-33.

Plants grown in nutrient solutions containing four concentrations of nutrients revealed following interactions before flowering. Mn, Fe, and Cu showed synergistic effects between N and P, P and Cu, and P and Fe; antagonistic effect between N and K, N and Zn, K and Mg, and K and S; and either synergistic or antagonistic interaction between Zn and B, and Mn and Mg (Lima and Malavolta, 1997).

3. Nutrient–Sweet Glycoside Relationship

There is a close association between nutrient supply and stevioside accumulation as evident from the studies all over the world. Though the requirements of micronutrients are lesser than macronutrients, experiments conducted in nutrient solutions indicated that Boron supplied at 5 ppm registered higher contents of stevioside and rebaudioside (Sheu *et al.*, 1987). Among secondary nutrients, only severe Ca deficiency caused reduction in the glycoside concentration (Filho *et al.*, 1997b). Besides, the role in growth and development, deficiencies of K, Ca, and S decreased the concentration of stevioside in the plant on dry weight basis while all deficiencies, except that of P, decreased the stevioside content in the plant (Utumi *et al.*, 1999). Supporting these results, research at Egypt showed a gradual and significant increase in stevioside content as nitrogen fertilizer increased from 10 to 30 kg N to the tune of 1.99% at the higher dose (Allam *et al.*, 2001).

E. CROP–WEED COMPETITION AND WEED MANAGEMENT

Stevia has a poor capacity to compete with weeds during the initial growth period and weeds are the principal competitors in limiting crop establishment and ultimately the yield. Furthermore, weeds make harvesting more difficult and increase weed seed build up in the soil. Cultural methods of weed control have always been important in the crop establishment process. Slow initial seedling growth rate (Shock, 1982) has been observed to accelerate weed competition. Weeds like *Ageratum houstonianum*, *Borreria alata*, *Digitaria* sp., *Eleusine indica*, *Erechtites valerifolia*, *Erigeron sumatrensis*, *Galinsoga parviflora*, and *Sida rhombifolia* were reported to be present in *Stevia* culture (Basuki, 1990). For these reasons, weed management plays a vital role in good crop management practices. Some natural means of weed management, such as higher plant densities, have been attempted (Basuki, 1990). They demonstrated that high plant density

(2 lakh ha⁻¹) combined with black plastic mulch provided effective control of weeds. The crop requires weed control at the early stages. Notwithstanding to this fact, work on weed management is lacking in literature.

Though there is a great deal of interest in organic cultivation, need for chemical weed management measures cannot be kept off. The choice of herbicide will depend upon the weed spectrum associated with the crop. There is a report that *Stevia* can tolerate trifluralin (Andolfi *et al.*, 2002; Katayama, 1978). At Palampur, India, crop planted during June experienced severe weed competition due to poor crop establishment (Ramesh, personal communication). This was exacerbated due to heavy rains. There is no published evidence regarding safe herbicides for *Stevia*.

F. WATER REQUIREMENT

The knowledge of water requirement of crops in different growing phases elicits higher crop yield and rational use of water resource. In natural habitat, it occurs in areas where the sites are continuously moist but not subjected to prolonged inundation. *Stevia* usually occurs on locations with high level of underground water or with continually moistened soil. It does not require frequent irrigation, though it is susceptible to moisture stress (Shock, 1982). It indicated that the crop prefers moist soil. For economic crops of *Stevia*, irrigation is necessary (Donalisio *et al.*, 1982). The plant has poor tolerance to pH, so it should not be grown with poor quality water (Shock, 1982). Plant growth was optimal at water content in soil of 43.0–47.6%. The average water requirement per day is 2.33 mm plant⁻¹ (Goenadi, 1983). Therefore, to secure optimum water relations for *Stevia* plants is one of the factors closely connected with its cultivation (Cerna, 2000). It requires liberal watering after transplanting, and before and after harvesting of the leaves (Andolfi *et al.*, 2002). The average crop evapotranspiration (Ete) was measured as 5.75 mm day⁻¹, and water consumption was high during the entire cycle. Irrigation at 117% of Ete was 13% better than 100% Ete in terms of *Stevia* yield (Fronza and Folegatti, 2002a). Evapotranspiration during the cycle was divided in to 3 parts: 6.66 mm day⁻¹ (0–25 days), 5.11 mm day⁻¹ (26–50 days), and 5.49 mm day⁻¹ (51–75 days) at Brazil (Fronza and Folegatti, 2002b).

The crop coefficient value (K_c) is the ratio between actual Ete to potential Ete. This could be used as a parameter to judge water requirements. Gonzalez (2000) had reported a crop coefficient value of 0.25 from 0 to 25 days, 0.56 from 26 to 50 days, and 0.85 from 51 to 80 days in Paraguay, whereas Fronza and Folegatti (2003) obtained 1.45, 1.14, and 1.16 at Italy for the said phases, respectively.

G. SOIL REQUIREMENT

The occurrence of *Stevia* on acid, infertile, sandy, or muck soils with ample supply of water is consistent with observations of plant performance under cultivation (Shock, 1982). The plant can be grown in a wide range of soils but has poor tolerance to salinity and so it should not be grown in saline soils (Chalapathi *et al.*, 1997b). This occurs on the edges of marshes or in grassland communities on soils with shallow water tables, the soils are typically infertile acid sands or mucks. *Stevia* will grow well on a wide range of soils given a consistent supply of moisture and adequate drainage. *Stevia* grows naturally on infertile, sandy acid soils with shallow water tables. This is normally in areas like the edge of marshes and grassland communities (Lester, 1999). But this can also grow in grasslands, scrub forests, and alpine areas (European Commission, 1999).

H. HARVEST

The optimum time of harvest depends on the cultivar and growing season. Leaves are harvested about 4 months after planting by cutting the plants at about 5–10 cm above the soil level (Donalisio *et al.*, 1982). This must however, the maximum crop biomass stage (Fig. 9), otherwise yield reduction is possible (Shuping and Shizhen, 1995). Since the crop is highly sensitive to low temperature, in cold areas, crop may be harvested before or at onset of winter (Columbus, 1997).

During flowering, stevioside dissipates from leaves (Bian, 1981; Hoyle, 1992), thus leaves should be harvested at the time of the flower (Figs. 10 and 11) emergence (Dwivedi, 1999) or before flowering (Barathi, 2003).

I. GROWTH REGULATORS

1. Foliar Application

The most effective preparation for increasing the concentration of stevioside in leaves was application of Humiforte (synthetic amino acids, N, P, K, and trace elements) in combination with aminol (amino acids and N). However, Maletran (lactic and anthranilic acids) gave the highest biomass of micropropagated plants under field conditions (Acuna *et al.*, 1997). The best growth (root, stem, leaf, and whole plant fresh weight) was observed in the third harvest due to Gibberellic acid (GA_3) at 50 mg liter⁻¹ treatment.



Figure 9 A field view of luxuriant *Stevia rebaudiana* at IHBT, Palampur, India.

No trends were observed in the 10 and 20 mg GA₃ liter⁻¹ treatments. However, the best overall growth was exhibited by the control (Stefanini and Rodrigues, 1999).

2. Tissue Culture

The combination of naphthalene acetic acid (NAA) and benzyl adenine (BA) @ 0.1–0.2 mg liter⁻¹ was found to induce shoot formation in *Stevia* explants. Further, the addition of GA to callus and suspension cultures resulted in a significant increase in their fresh weight (Bondarev *et al.*,



Figure 10 *Stevia* at budding stage.

1998). Whereas growth regulators depress the content of steviol glycosides, however, the ratio of glycosides remained the same (Bondarev *et al.*, 2003a).

J. SEED PRODUCTION

Seed yield up to 8.1 kg ha^{-1} is possible (Carneiro, 1990). However, the climatic requirements, of day length and temperature, are different for maximum vegetative production and for maximum flowering and seed production (Hoyle, 1992) since the crop is triggered to flowering under long day conditions. It is not the only determinant governing seed production but nutritional requirements are also higher. Seed production in the northern hemisphere would be best suited between 20 and 30° N latitude. The crop could be transplanted in February–March and seed collected in late summer. The test weight of *Stevia* seeds range between 0.15 and 0.30 g (Brandle *et al.*, 1998) and 0.30 and 0.50 g under Palampur conditions (Ramesh, personal communication).

K. CORRELATION STUDIES

Several authors studied the yield dependence on various growth parameters as well as stevioside content (Brandle and Rosa, 1992; Buana, 1989; Buana and

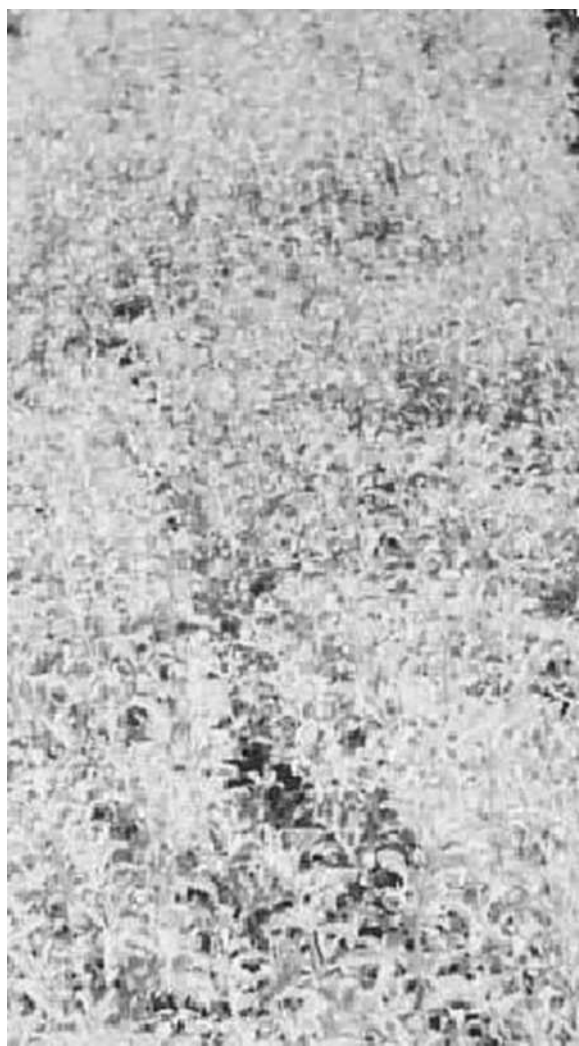


Figure 11 A field view of *Stevia* flowering.

Goenardi, 1985; Chalapathi *et al.*, 1998, 1999a; Nishiyama *et al.*, 1991; Shu and Wang, 1988; Shyu *et al.*, 1994; Truong *et al.*, 1999; Utumi *et al.*, 1999).

Plant height and leaf number at second and fourth week after planting was positively correlated with *Stevia* biomass production at 30 DAT in a greenhouse experiment (Buana and Goenardi, 1985). In another study, plant height neither had any close relationship with production nor with leaf number or branch number in the first 4 weeks (Buana, 1989). A positive correlation

between total soluble carbohydrate content and stevioside content was established by Nishiyama *et al.* (1991). Stevioside concentrations were uncorrelated to yield or leaf:stem ratio (Brandle and Rosa, 1992). Further, dry leaf yield was correlated with leaf size and thickness, content of rebaudioside A was correlated with rebaudioside C, and rebaudioside A to stevioside ratio was highly correlated with leaf thickness (Shyu *et al.*, 1994).

The dry yield of *Stevia* was positively and significantly correlated with plant height, number of branches, leaves per plant, and dry matter accumulation. About 96.88% of the total variation in dry leaf yield was explained by a linear function of these four characters (Chalapathi *et al.*, 1998). The number of branches, and yield of fresh and dry stem and leaf, was more variable than the number of leaf pairs, number of nodes before transplanting and at harvest, plant height at transplanting and leaf length at harvest. The characters most closely related to yield were fresh and dry weights of leaves and stems. Step-by-step regression showed that leaf dry weight/plant had the greatest effect on yield (Shu and Wang, 1988). Stevioside content is influenced by both leaf surface and number of roots; however, the former has greater influence on stevioside content than number of roots as evident from the correlation coefficient (Truong *et al.*, 1999), since the chemical content of last fully expended leaf pairs was well correlated with plant nutrient status (Utumi *et al.*, 1999).

L. BIOTIC STRESSES

Earlier, diseases like powdery mildew (*Erysiphe cichoracearum* DC), Damping off (*Rhizoctonia solani* Kuehn.), and Stem rot (*Sclerotium dephini* Welch.) were reported by Thomas (2000). Two fungal diseases *Septoria steviae* and *Sclerotinia sclerotiorum* were reported in *Stevia* grown in Canada (Chang *et al.*, 1997; Lovering and Reeleder, 1996; Reeleder, 1999). Occurrence of stem-rot disease was recorded by 0.1% in the crop field at Palampur, India (Megeji *et al.*, 2005). Incidence of insects like aphids and white flies were observed in the experimental field at IHBT, Palampur, but these were below the threshold level. Similarly, attack of insects like aphids, mealy bugs, red spider mites, and whiteflies were reported by Thomas (2000).

M. CROP PRODUCTIVITY

Stevia is a semiperennial species, which can be maintained up to 5–6 years, with 2 or 3 harvests per year. Earlier, Bridel and Lavielle (1931a,b,c) and Metivier and Viana (1979a) reported a stevioside yield of 60–65 and 72 g kg⁻¹ dry leaf, respectively. In terms of economic biomass productivity, the dry leaf yield in the natural habitat, Paraguay, was between 1500 and 2500 kg ha⁻¹

under dry land conditions and around 4300 kg ha⁻¹ with irrigation per year (Jordan Molero, 1984).

Leaf yields of 3000 kg ha⁻¹ with a stevioside concentration of 105 mg g⁻¹ equivalent to 66.2 ton ha⁻¹ of sugar was obtained at Canada (Brandle and Rosa, 1992).

In Japan, 1 or 2 harvests per year is possible with a dry leaf yield of 3000 and 3500 kg ha⁻¹ in the first year, 4000–4500 kg ha⁻¹ in the second, 4000–6000 kg ha⁻¹ in third, diminishing to 4000 kg ha⁻¹ in the fourth year (Sunk, as quoted by Taiariol, 2004).

Under agro-climatic conditions of Palampur, first harvest is taken at 90–110 days after transplanting during June–July. Subsequently, second harvest is taken after 60–75 days of the first harvest in early September at the time of flower bud initiation. In case of late transplanted crop grown for single cut, harvesting is done after 3–4 months of transplanting and continues till flowering begins, because the maximum sweetener in the leaves is until the plant bears flowers. Perennial crop may continue up to 4 years, once planted, in the same field. Life span of the crop is reported to be 7–8 years and herb yield increases up to 4 years. Maximum amount of leaves are produced in the third or fourth year. Flowering of the plant should be avoided and pinching of the apical bud should be done to enhance bushy growth of the plant with side branches. In the first year, average fresh biomass yield of 15–20 ton ha⁻¹ was obtained out of two harvests and increased in subsequent years up to 20–30 ton ha⁻¹. An average dried leaf yield of 17, 20, 23, and 25 q ha⁻¹ could be produced from this total biomass yield in the first, second, third, and fourth years, respectively (Singh and Kaul, 2005).

VII. CHEMISTRY AND QUALITY

The sweetness in *Stevia* is attributed to the presence of *ent*-kaurene diterpene glycosides, which are water soluble (Duke and deCellier, 1993; Lester, 1999) and 300 times as sweet as cane sugar (Metivier and Viana, 1979b). *Stevia* leaves accumulate a mixture of at least eight different glycosides derived from the tetracyclic diterpene steviol (Brandle *et al.*, 1998). The leaves contain stevioside, rebaudioside A, B, C, D, and E, dulcoside A, and steviolbioside. The sweetening potency (sucrose = 1) was 250–300, 350–450, 300–350, 50–120, 200–300, 250–300, 50–120, and 100–125, respectively (Crammer and Ikan, 1986). These products taste intensely sweet; for example, rebaudioside A has been shown to be up to 320 times sweeter than sucrose on a weight basis (Phillips, 1987). Stevioside is a white amorphous powder present in leaf and stem tissue, was first seriously considered as a sugar substitute in the early 1970's (Kinghorn and Soejarto, 1985). The

sweetness in the leaves is two times higher than that of inflorescence (Dwivedi, 1999). Steviolbioside 2, rebaudioside A4, B5, C6, D7, E8, and F9, and dulcoside A10 are other compounds present but in lower concentration (Kennely, 2002; Starrat *et al.*, 2002). This is an alternate to artificial sweeteners such as aspartame or sodium saccharin. There is no report of ill effect on human health in over 1500 years of continuous use by Paraguayans. In Japan (the biggest consumer market), there have been no reports of side effects. Reports on antifertility (Planas and Kuc, 1968) and its metabolic byproducts like steviol being highly mutagenic (but no confirmative reports are available for harmful effect on using this plant; Brandle and Rosa, 1992) leads to a controversy on safety concern of this plant in humans. The sweet compounds pass through the digestive process without chemically breaking down; making *Stevia* safe for those who need to control their blood sugar levels (Strauss, 1995). A more detailed discussion on biosynthesis, toxicity, metabolism, and nutritional implications of stevioside was reviewed by Geuns (2003), which contains 74 references. He concluded that most toxicity tests performed on stevioside have been negative and the use of purified stevioside as a food additive appears preferable from public safety point of view. The conclusion is that *Stevia* and stevioside are safe when used as a sweetener.

VIII. RESEARCH NEEDS

Good agricultural practices (GAP) of *Stevia* cultivation are the need of the hour. An integrated approach by a team of multidisciplinary scientists is required, leading to good manufacturing practices (GMP) of desired quality end product from this crop. Use of *Stevia* is intimately tied to two major sweet glycosides, stevioside and rebaudioside A, because of the prominence of these compounds in this plant. Therefore, research should be directed toward the improvement of stevioside and rebaudioside A through management and crop improvement strategies. *Stevia* gives a new direction for the farming community, businessmen, and also the researchers. The possible issues are enhancing the specific enzyme responsible for the production of these glycosides so that their yield gets enhanced. Quality of sweetness is also dependent on higher proportion of rebaudioside A to stevioside in the extracted composite powder.

In many countries, this is a crop of recent domestication. Therefore, agronomic considerations should be of high priority to utilize its maximum potential. Under subtemperate climate prevailing in mid hills of India and analogous regions of the world, growth of seedlings take longer time and vegetative propagation is restricted due to nonavailability of actively

growing shoots. This leads to delay in large-scale commercial plantation. Studies on production techniques and planting through rootstock are needed.

Water management component is considered to be critical, since the water resources are shrinking day by day. Integrated crop management comprising of weed, insect, disease, and nutrient management, should be inbuilt as a part of GAP. As a system study, the suitability of this crop in the traditional cropping systems is another determinant to avoid excess production. This complete packages of production technology will make the *Stevia* cultivation socially acceptable, cheaper, and economically viable.

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ASSESSING SOIL FERTILITY DECLINE IN THE TROPICS USING SOIL CHEMICAL DATA

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Soil fertility decline is perceived to be widespread in the upland soils of the tropics, particularly in sub-Saharan Africa. Most studies have used nutrient balances to assess the degree and extent of nutrient depletion; these have created awareness but suffer methodological problems as several of the nutrient flows and stocks are not measured. This chapter focuses on the assessment of soil fertility decline using soil chemical data (pH, organic C, total N, available P, cation exchange capacity (CEC), and exchangeable cations) that are routinely collected in soil surveys or for the assessment of fertilizer recommendations. Soil fertility decline can be assessed using a set of properties from different periods at the same site or from different land-use systems with the same soils. The former is easier to interpret; the latter can be rapidly collected but differences may be due to inherent differences and not have resulted from soil management. This study provides an analytical framework for the assessment of soil fertility decline and shows pitfalls and how they should be handled. Boundary conditions are presented that could be used in future studies on soil fertility management and crop productivity in the tropics.

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I. INTRODUCTION

Crops remove nutrients from the soil through the agricultural produce (food, fiber, wood) and crop residues. This may result in declining soil fertility if replenishment with inorganic fertilizers or manure is inadequate. A decline in soil fertility implies a decline in the levels of soil organic C, pH, CEC, and plant nutrients. Soil fertility decline includes nutrient depletion (larger removal than addition of nutrients), nutrient mining (large removal of nutrients and no inputs), acidification (decline in pH and/or an increase in exchangeable Al), the loss of organic matter, and an increase in toxic elements such as aluminum (Hartemink, 2003).

Several studies in the 1990s indicated that soil fertility decline is a problem in many tropical countries and, particularly, in sub-Saharan Africa (Brand and Pfund, 1998; Folmer *et al.*, 1998; Hartemink, 1997a; Henao and Baanante, 1999; Nandwa and Bekunda, 1998; Pieri, 1989; Probert, 1992; Rhodes, 1995; Stoorvogel and Smaling, 1990; van der Pol and Traore, 1993). Most of these studies were based on nutrient balances or budgets in which fluxes and pools were estimated from published data, data derived from pedotransfer functions, or some other method. The studies were conducted mostly on the country or subcontinental level. One influential study was conducted by Stoorvogel and Smaling (1990) who calculated budgets for N, P, and K for the arable soils of 38 countries in sub-Saharan Africa for the years 1983 and 2000. The nutrient inputs were less than the outputs in nearly all countries in sub-Saharan Africa.

In recent years, there has been criticism of such studies—on the way the study was conducted as well as on the extent and impact of soil fertility decline (Hartemink and van Keulen, 2005). Some researchers consider nutrient depletion to be a serious problem (Koning and Smaling, 2005; Muchena *et al.*, 2005) hindering agricultural development; others argue that the problem is exaggerated and that farmers have found ways to deal with dwindling soil fertility (Fairhead and Scoones, 2005; Mortimore and Harris, 2005; Niemeijer and Mazzucato, 2002). They see evidence in farmers' practices and yield patterns over time that do not match the widely perceived soil fertility decline.

These contradictions are in part due to lack of fundamental knowledge, differences in perceptions, differences in research methodology, and the scale of observation. In some areas, soil fertility has declined because of reduced fallow period in shifting cultivation systems and little or no inorganic fertilizer inputs. In other areas, soil fertility may have been maintained or improved at the expense of land elsewhere, that is, through biomass transfer. Where such differences are explored in more detail, there are complex explanations including non-agronomic aspects like infrastructure, access to markets, political stability, land tenure, and investments. A detailed analysis of causes and effects requires a multidisciplinary effort of which soil scientists need to be part. However, they need to be equipped with arguments and data to feed the discussion. A rigorous analysis and assessment of soil fertility decline requires solid scientific methodologies and approaches.

As mentioned, most of the discussion has been fueled by nutrient balance studies. Relatively little use has been made of soil chemical data from different periods or land-use systems, although such data are available from soil surveys, soil fertility evaluations and fertilizer programs. They may be available from long-term experiments of which there are several in tropical regions (Bekunda *et al.*, 1997; Greenland, 1994a; Laryea *et al.*, 1995; Pieri, 1995; Singh and Goma, 1995; Smyth and Cassel, 1995; Steiner and Herdt, 1993). Recent rapid assessment of soil fertility properties using spectroscopy (Shepherd and Walsh, 2002; Shepherd *et al.*, 2003) greatly increase the amount of data on tropical soils. Moreover, there is increase access to soil testing databases through the internet (Motavalli *et al.*, 2002). Although there are several techniques to derive soil properties using pedotransfer functions or soil inference systems (McBratney *et al.*, 2002; Pachepsky and Rawls, 2005), the need remains for reliable data to validate and further develop our models and increase the understanding of soil behavior and human-induced soil changes.

This chapter reviews the major aspects of using soil chemical data for the assessment of soil fertility decline in soils of the tropics. Such assessment is needed to adequately address the issue of sustainable land management for increased food production and the alleviation of hunger in many parts of the

tropics. To assess soil fertility decline, it is necessary to define the spatial and temporal boundaries of the systems under study, the data types that are available and their spatial and temporal variation, and proper soil sampling procedures and analysis to obtain meaningful results. In the final part of this paper interpretation of results including resilience and reversibility is discussed.

II. CHANGES IN SOIL CHEMICAL PROPERTIES

The total amount of a nutrient in the soil declines when the output exceeds the input over a given period of time, soil depth, and at a certain location. To ascertain whether nutrient levels, pH, or soil organic C have declined, spatial and temporal boundaries must be chosen. A spatial boundary is, for example, the plot. A temporal boundary might be the period during which the plot was cultivated. When such boundaries are properly set it is possible to conclude, for example, that the soil fertility has declined in a wheat field between 1980 and 2005. Such a simple conclusion involves various complicated steps and these are discussed later.

The soil can be considered a box from which nutrients are removed (output) and in which nutrients are entered (input). The box is the pool of nutrients (nutrient stock) whereas input and output of nutrients are fluxes or flows. This approach has been adopted in agricultural and ecological research since the seminal work of Nye and Greenland (1960). When the pool is measured over a period of time, no changes may have occurred: the soil is in a steady-state condition in which the properties are in dynamic equilibrium (Yaalon, 1971). Such a condition is exceptional and there is increasing evidence that, even under natural conditions, nutrient losses occur (Poels, 1989; Stoorvogel *et al.*, 1997). The steady-state condition does not imply that losses are absent and provides no information on the environmental impact of a land-use system.

The second possibility is that the pool has increased due to nutrient inputs exceeding nutrient outputs, or because the output of nutrients is decreased whereas the inputs remained the same, or a combination of the two. Higher inputs may also mean higher outputs but, as long as total input exceeds output, the soil fertility builds up. This has occurred in the Pleistocene sandy areas of the Netherlands, Germany, and Belgium where plaggen soils, or Anthrosols, developed through centuries of applications of a mixture of manure, sods, litter, and sand (Pape, 1970). Similar soils occur in Russia (Giani *et al.*, 2004) and several other European countries. The buildup of nutrients continues to the present day through high applications of animal manure and inorganic fertilizers to agricultural soils of several Western

European countries (de Walle and Sevenster, 1998). As a result, nutrients may be leached into surface and groundwaters potentially causing environmental problems. This is an important drive for manure legislation and precision agriculture (Neeteson *et al.*, 2002; Pierce *et al.*, 1999; Robert, 2002). There are also several examples in the tropics where soil fertility has built-up as a result of long-term applications of organic materials (Lima *et al.*, 2002; Sandor and Eash, 1995) including household waste and manure (FAO, 2001b).

The third possibility is that the pool decreases and soil fertility declines. This occurs when nutrient output exceeds input over a given period due to increasing outputs when inputs remain the same. This may occur, for example, when there is a sudden increase in the rate of erosion or leaching because of high rainfall events. Decreased inputs may also imply decreased outputs, but the net difference between nutrient input and output determines whether there is a decline in soil fertility.

A. ADDITIONS, REMOVALS, TRANSFORMATIONS, AND TRANSFERS

Factors affecting soil fertility decline are essentially the same as those in pedogenesis: additions, removals, transformations, and transfers. In natural ecosystems, additions of nutrients occur through atmospheric deposition, biological nitrogen fixation (BNF), and sedimentation whereas removals include leaching, gaseous losses (denitrification, volatilization), and through soil erosion. In agricultural ecosystems, nutrients are also removed with the crop yield and crop residues, whereas nutrients may be added with animal manure, inorganic fertilizers or other amendments. There are important differences in the rates of soil processes between natural and agricultural ecosystems. In agro-ecosystems there is periodic disturbance, including tillage, weeding, and the application of soil amendments, which determine the additions and removals of nutrients. Also nutrient removal through erosion and leaching is generally higher in agricultural ecosystems (Logan, 1990).

Some of the additions and removals in a nutrient balance are difficult to quantify. For example, leaching is governed by intrinsic soil properties (porosity) in relation to climate (periodic excess of rainfall over evapotranspiration), cropping systems (rooting system), and soil management (inorganic fertilizer applications, organic matter content). The fact that several interacting factors are involved explains why quantifying leaching losses is difficult (Addiscott, 1996). Denitrification is also difficult to quantify, but it is generally assumed to be low in upland soils of the humid tropics (de Klein *et al.*, 2003; Grimme and Juo, 1985). Volatilization has been studied particularly in relation to the efficiency of N fertilizers (Harrison and Webb, 2001; Raun and Johnson, 1999). Quantification of BNF has received much attention in

Table I
Additions, Removals, Transformations, and Transfer of Nutrients in Soils Under Natural and Agricultural Ecosystems

	Factors in natural ecosystems	Additional factors in agricultural ecosystems
Additions (input)	Dust, nutrients in the rainfall, symbiotic and asymbiotic N fixation, sedimentation	Inorganic fertilizers, organic manure
Removals (output)	Leaching, volatilization, denitrification, erosion	Removal of nutrients in economic produce and crop residues
Transformations	Mineral weathering, organic matter decomposition, fixation	None
Transfer	Deep uptake, clay eluviation and illuviation	None

tropical ecosystems as it is considered to be a viable way of increasing the N status of soils in the tropics (Boddey *et al.*, 1997; Giller *et al.*, 1997; Greenland, 1985).

A transformation of nutrients is a change from an organic into an inorganic form or *vice versa*. Mineral weathering releasing cations and organic matter decomposition releasing N, P, and S are transformation of nutrients. Other examples are P fixation by sesquioxides and allophane, or N immobilization due to additions of organic matter with high lignin content. These are neither a loss nor an output but a transformation into a form that is not directly available for plant growth. The transformations may imply that the nutrient is not available for crop production for long periods (>100 years).

Transfer of a nutrient refers to replacement within the soil like deep uptake by tree roots, or the eluviation of organic matter or K-rich clay minerals into deeper soil layers. Both deep uptake and eluviation are not a removal of nutrients but a transfer of nutrients to other soil layers.

Table I summarizes additions, removals, transformations, and transfer of nutrients in soils under natural and agricultural ecosystems. As mentioned, the rate and direction of these processes is different under natural ecosystems compared to agricultural ecosystems.

B. SPATIAL BOUNDARIES

The boundaries of the box need to be defined before measurements are made and soil fertility changes can be assessed. If the box has unrestricted depth, deep uptake and leaching are a transfer of nutrients but gaseous

losses and runoff are outputs because nutrients depart from the box. Replenishment of nutrients can take place only through inputs, that is, animal manure, inorganic fertilizers, and biological nitrogen fixation. Deep uptake can be considered as a nutrient input only when the box has a horizontal boundary at some depth. Deep uptake is important not only in agricultural systems with a tree component but also in natural forests (Hartemink *et al.*, 1996; Lehmann *et al.*, 2004; Poszwa *et al.*, 2002).

In addition to the depth of the box, the width and length are important boundaries. If the box is large, then losses of nutrients by erosion and subsequent deposition in a lower position of the landscape are a transfer of nutrients. The same applies for nutrients transported by subsurface flow. When soil fertility changes are evaluated for an entire catchment, it may be found that nutrients in the upper part of the catchment are being lost whereas there is net gain in the lower part. The net changes within the catchment may be nil, whereas differences between the sampling sites are large.

The transfer of nutrients from one area or spatial scale to another is natural, as for example, the deposition of nutrient-rich sediments in alluvial plains and river deltas, or the Harmattan dust from West Africa which reaches Europe (Simonson, 1995). It shows that the spatial scale or boundary affects the conclusions of a soil fertility decline evaluation. There are methods available to use soil fertility data at different scales (McBratney, 1998; Stoorvogel and Smaling, 1998), but universally applicable scaling rules are to be developed.

C. TEMPORAL BOUNDARIES

Monitoring soil chemical properties implies that observations are made at different time steps. Soil fertility data can be compared for the different times, and it provides some information on what has happened between the two sampling periods (Hartemink, 2003). If there are only two sampling periods then no information is collected on the exact pattern of change (see Section VII). For example, in shifting cultivation systems the level of exchangeable cations may be low prior to cutting the fallow vegetation but increases when the vegetation is cleared and cations are released from the burning of the biomass (Lal, 1986; Nye and Greenland, 1960). The higher levels of exchangeable cations are usually followed by a rapid decrease due to losses and crop uptake (Sanchez *et al.*, 1983). In such dynamic systems, different conclusions are reached when measurements and observations are made at different time steps.

The soil science literature is flooded with short-term observations, by which transient phenomena can be missed or misinterpreted (Pickett, 1991).

In general, observations made over a long period allow more rigorous analysis. If, however, long-term observations also imply large time steps, it may mask what has happened during the period of observation. Therefore, the best approach is long-term monitoring with relatively short time steps (Hartemink, 2003).

Also the soil property chosen and the techniques used for its analysis affects the assessment of soil fertility decline. Changes in the microbial population occur within hours or days, whereas a significant difference change in CEC may require years of cultivation between the measurements. A related problem is that some soil parameters fluctuate during the day or between seasons and detecting changes may therefore be confounded with the natural variability (see Section IV).

III. DATA TYPES

Some soil degradation features like soil erosion and salinization can be observed and assessed by remote sensing (Cresswell *et al.*, 2004; Goossens and Van Ranst, 1998; Howari, 2003; Servenay and Prat, 2003). Such techniques cannot yet be used to adequately assess a decline in soil nutrient levels. In the literature on soil fertility decline, three different data types are used to assess soil chemical changes caused by agricultural production systems: expert knowledge, nutrient balances, and the monitoring of soil chemical properties (Hartemink, 2006). Some of these data can be collected relatively easily, whereas others require long-term commitment. Each data type has specific advantages and disadvantages; the type of data collected is determined by the research plan, and the financial conditions.

A. EXPERT KNOWLEDGE

Traditionally, soil science has used several qualitative measures of soil properties like soil color and field texture. Soil mapping also has qualitative aspects including the delineation of mapping units. Qualitative approaches have greatly contributed to our knowledge on soil resources and form the base for what can be called expert knowledge systems. Also farmers and other users of the land have expert knowledge about soils; this is loosely described as indigenous soil knowledge or ethnopedology (Barrios and Trejo, 2003; Krasilnikov and Tabor, 2003; Sillitoe, 1998; Warkentin, 1999; WinklerPrins and Sandor, 2003). Indigenous soil knowledge has different characteristics from knowledge gained by the scientific study of the soil.

Farmers' empirical knowledge is not soil process or data-oriented but yield or management-oriented (Bouma, 1993). Yield decline, as observed by a farmer could be caused by a variety of factors including soil fertility decline, adverse weather conditions, invasion of weeds, soil physical deterioration, or a combination of factors. Therefore, farmers' knowledge of soil fertility decline is difficult to interpret if not augmented by other types of data, like for example crop yield, weather conditions, or information on pests and diseases (Hartemink, 2003).

The annotated bibliography on ethnopedology Barrera-Bassols and Zinck (2000) lists more than 900 references and abstracts and the bibliography provides a wealth of information on how farmers perceive soil fertility. This perception is almost universally qualitative and, as mentioned, could be affected by many biophysical and management factors including the memory or political motives of the farmer. For example, in a land degradation study in Burkina, Faso Gray (1999) found that the perception of local farmers was frequently socially constructed and politically mediated. Perceptions of degradation were related to ethnic conflicts over land. Environmental development projects offered tangible benefits for farmers who perceive their resource base is degraded (Gray, 1999). In other words, it was beneficial to exaggerate the situation.

A good example of expert knowledge was the first approximation to assess and map soil degradation at a global scale—GLASOD (Oldeman *et al.*, 1991). More than 200 soil and environmental scientists worldwide were asked to give their opinion on the types, degrees, and areal coverage of human-induced soil degradation in their regions. Two categories of soil degradation were distinguished: the first relates to displacement of soil material (soil erosion by water and wind) and the second was soil deterioration *in situ* like chemical and physical degradation. GLASOD showed that loss of nutrients (i.e., soil fertility decline) was severe in Africa and South America but less a problem in the upland soils of Asia.

Expert knowledge is largely qualitative, may have political motives, and is not useful for the quantitative assessment of soil fertility decline. Nonetheless, such knowledge can be useful in selection of sampling sites or for additional information. There are examples where expert knowledge was used to investigate long-term changes in soil fertility (Peters, 2000; Sillitoe and Shiel, 1999).

B. THE NUTRIENT BALANCE

Another approach to investigate changes in soil fertility is the nutrient balance approach, which in essence is an accounting procedure. Inputs

(e.g., fertilizers, manure) are compared to nutrient outputs (crop removal, leaching etc.) over a given time span that is mostly one cropping season or 1 year. Nutrient balances are a convenient and biologically meaningful way to investigate what is known about a system's biogeochemical cycles. It can give insight into the processes that regulate nutrient cycling, and nutrient balances may help to formulate system management decisions and direct the course of research (Hartemink, 2005; Robertson, 1982). In several countries, nutrient balance studies have been used in studies on food crop production and the maintenance of the soil resource base (Johnston and Syers, 1998).

Nutrient balances have been used mostly at national or supranational scale, although some studies have been conducted at the district scale (Smaling *et al.*, 1993; van der Pol and Traore, 1993) or farm scale (Shepherd *et al.*, 1996; Van den Bosch *et al.*, 1998; Wortmann and Kaizzi, 1998). In the national or supranational studies, existing soil data are combined with pedotransfer functions to estimate the decline in soil fertility at a given location (Stoorvogel and Smaling, 1990). This is a mechanistic modeling approach in which expert knowledge is also important. Such studies are not to replace soil-monitoring but to be seen as the best possible way of getting the most out of available data (Hartemink, 2003). The outcome of such studies provides qualitative, comparative, and spatial information on the difference between nutrient input and output. The maps may raise more awareness amongst the public and policy makers than facts and figures collected by soil monitoring.

1. Methodological Problems

Nutrient balance studies in tropical regions have been influential—both agronomically and politically—but there are some methodological problems (Faerge and Magid, 2004; Sheldrick *et al.*, 2002). Some of the input and output data in the supranational or national nutrient balances were easily obtained whereas others were hard to quantify or relied heavily on FAO soil and crop databases, which are not always accurate. Yield data from FAO databases were multiplied by standard nutrient uptake data of crops (Cooke, 1982). Nutrient uptake data are variable. For example, Hartemink (1995) showed that, based on 11 literature sources, nutrient removal of *Agave sisalana* varied from 27–33 kg N ha⁻¹, 5–7 kg P ha⁻¹, and 59–69 kg K ha⁻¹ per ton of fiber produced. This variation may be attributed to differences in sampling techniques, sampling period, inherent soil conditions, fertilizer applications, and analytical methods. Multiplying the uptake values with the range of yields in the FAO databases gives a range in calculated nutrient removal, which is often the largest factor in nutrient balance studies.

In the absence of sufficient data, several factors were estimated using pedotransfer functions. Denitrification in the sub-Saharan Africa nutrient balance study was estimated from studies conducted in Puerto Rico (Dubey and Fox, 1974), which may not hold for the range of soils and climates in Africa. Nitrogen inputs by BNF and wet deposition were estimated from average annual rainfall whereas such inputs vary greatly between regions and seasons (Clark *et al.*, 1998; Walley *et al.*, 2001). Faerge and Magid (2004) concluded that the transfer functions in nutrient balance studies tend to overestimate losses and that no check has been made to assess whether the modeled losses are consistent to empirical measurements. In the supranational study of Stoorvogel and Smaling (1990), nutrient depletion in Gambia for the year 2000 was estimated to be -17 kg N , -3 kg P , and -24 kg K ha^{-1} . Henao and Baanante (1999), who used a similar approach to Stoorvogel and Smaling, estimated the nutrient balance for Gambia over the years 1993–1995 to be: -30 kg N , -5 kg P , and $-18 \text{ kg K ha}^{-1} \text{ year}^{-1}$. However, a nationwide soil fertility evaluation in 1992 and 1998 showed that available P and exchangeable K levels had actually increased in soils under the main food crops (Peters, 2000). This shows that nutrient balance studies need to be combined with field measurements for accurate assessment of soil fertility decline.

When all factors in a nutrient balance are considered and accumulated errors are calculated, the difference between the nutrient outputs and inputs may show a wide range of values. For example, if the sum of nutrient inputs is on average 150 kg ha^{-1} (range: $125\text{--}175 \text{ kg ha}^{-1}$) and the sum of nutrient output is on average 200 kg ha^{-1} (range: $175\text{--}225 \text{ kg ha}^{-1}$), the difference between the averages is -50 kg ha^{-1} . If the range of values is considered, the difference could be 0 or as large as -100 kg ha^{-1} . There are, however, no nutrient balance studies in which standard errors or the range of values of different factors are given. Moreover there may be a difference between years depending on the weather and other factors. As a result, the average annual nutrient balance may be negative, but it may largely differ between years (Sheldrick *et al.*, 2003).

An overview of sources of biases in nutrient budgets was prepared by Oenema and Heinen (1999). Possible biases in the budget could be personal (conceptual interpretation and simplification of the system and its flows), sampling biases of nutrient pools and flows, data manipulation biases through generalization, averaging, and upscaling. Biases may also be due to fraud that could occur when nutrients budgets are being used as a policy instrument to enforce a nutrient management strategy with possible economic consequences for farmers and other stakeholders.

It should be mentioned that the authors never ignored the inherent methodological problems with the nutrient balance but justified the overall simplifications because of the lack of data (Smaling, 1993).

2. Recent Efforts

Most nutrient balance studies have been at the macro level (e.g., national, supranational) or the micro level (farm). They have provided data for decision makers and farmers. They have ignored an intermediate level that is operating at the level of a province, agro-ecological zone, or agro-economic system and this level has been termed the mesolevel (FAO, 2004). Compared with earlier studies, the mesolevel approach by FAO is more quantified and used more accurately, which were not available in the early 1990s. Most of the input and output factors could be better quantified and also dynamic landscape models were used. The results, however, are not very different. For three countries (Ghana, Kenya, Mali) nutrient depletion rates were similar to those presented in the studies from the 1990s. However, linking the results with food security or poverty maps using GIS appeals to policy makers (FAO, 2004). Further quantification of the nutrient balance allows for spatially explicit assessment of soil fertility decline at a range of scales, and the well-quantified approach of this study appears to be the way ahead for future nutrient balance studies.

C. MEASURED CHANGE IN SOIL CHEMICAL PROPERTIES: TYPE I DATA

Two approaches have been used to investigate changes in soil chemical properties. First, soil properties can be monitored over time at the same site, which is called chronosequential sampling (Tan, 1996) or Type I data (Sanchez *et al.*, 1985). Type I data show changes in a soil chemical property under a particular type of land-use over time. The original level is taken as the reference level to investigate the trend. It is most useful if trends are also followed under other land-use systems, for example, under cultivation, secondary regrowth, and natural forest over the same period; these data expose differences more accurately because natural forest ecosystems are not stable *per se*, especially under marginal conditions (Poels, 1987; Stoorvogel *et al.*, 1997).

Soil chemical data can be derived from stored samples that are analyzed at the same time as the newly collected soil samples, but the storage of soil samples may affect their chemical properties (Chapman *et al.*, 1997; Jones and Shannon, 1999; Slattery and Burnett, 1992). Alternatively, data from samples analyzed in the past can be compared to newly collected and analyzed soil samples provided analytical methods as well as laboratory errors are the same (see Section IV).

Type I data have been used for quantifying soil contamination by comparing soil samples collected before the intensive industrialization period with recent samples taken from the same locations (Lapenis *et al.*, 2000). Type I data are useful to assess the sustainability of land management

practices in the tropics (Greenland, 1994b), but few data sets exist because they require long-term research commitment and detailed recordings of soil management and crop husbandry practices (Hartemink, 2003).

D. MEASURED CHANGE IN SOIL CHEMICAL PROPERTIES: TYPE II DATA

In the second approach to investigate changes in soil chemical properties, soils under adjacent different land-use systems are sampled at the same time. This is called biosequential sampling (Tan, 1996), Type II data (Sanchez *et al.*, 1985), or “sampling from paired sites” in the soil science literature from Australia (Bramley *et al.*, 1996; Garside *et al.*, 1997). It has also been named the “space-for-time” method (Pickett, 1991) and the “inferential method” (Ekanade, 1988).

The underlying assumption is that the soils of the cultivated and uncultivated land are the same and that differences in soil properties can be attributed to differences in land-use and management. Obviously, this is not always the case; the uncultivated soil may have been of inferior quality and therefore not planted. Also, spatial variability may be confounded with changes over time (Sanchez *et al.*, 1985). Other confounding factors are differences in clay content, soil depth, or unknown history of land-use.

In ecology, Type II data studies have often proven to be misleading as functional parameters like nutrient availability and plant–animal interactions have been conspicuously underrepresented (Pickett, 1991). When carefully taken, however, Type II soil samples can provide useful information, and this sampling strategy has been followed in many studies investigating the effects of cultivation on soils.

Table II summarizes the different data types. Each data type has its own merits and drawbacks but the most comprehensive and effective characterization of soil behavior is obtained when all three data types are available.

E. MINIMUM DATA SETS

How much data are required and which soil attributes should be measured to assess that at a given location the soil fertility has declined? In the literature, these questions have been discussed in relation to the assessment of sustainable land management (Smyth and Dumanski, 1995) and in relation to such concepts as “soil quality” and “ecosystem health” (Doran and Parkin, 1996; Greer and Schoenau, 1997; Sposito and Zabel, 2003). Determining what data are to be included depends on the type of study and its objectives. For example, if the effects of continuous wheat on soil organic matter is to be examined, then measurements of soil organic C, light fraction, and particulate organic matter as well as mineralisable C, microbial

Table II
Data Types in Soil Fertility Decline Studies and Their Main Advantages and Disadvantages

Data type	Short description	Advantages	Disadvantages	Examples
Type I Chronosequential	Monitoring soil properties over time	Accurate, unequivocal, using existing data	Slow, contamination of monitoring sites, spatial and temporal variability, problems with soil sample storage, consistent laboratory procedures required, costly	Gray, 1999; Peters, 2000; Sobulo and Osiname, 1986
Type II Biosequential	Comparing soil properties under different land-use	Easy to obtain, rapid	Soils at sampling sites may differ, unknown land-use history of sites, spatial and temporal variability	Ayanaba <i>et al.</i> , 1976; Islam and Weil, 2000; Koutika <i>et al.</i> , 2000
Nutrient balance, nutrients budgets	Combination of existing data with pedotransfer functions or models	Using existing data, fairly rapid, indicative, appealing outcome	Several unknown or unmeasured fluxes and pools, hard to follow changes over time	Henao and Baanante, 1999; Stoorvogel and Smaling, 1990

Partly after Hartemink (2003).

biomass, and soil carbohydrates and enzymes should be included. Also bulk density and soil texture are needed. If it is to be determined whether leguminous crops enhance soil acidity, then the soil pH, buffering capacity, CEC, and exchangeable cations and soil acidity should be measured. These are, however, specific types of studies and for soil fertility decline studies the standard set of soil chemical properties suffice.

Soil organic matter is a key component of soil fertility (Chantigny, 2003; Woerner *et al.*, 1994). It is an essential soil property in soil fertility decline studies and Gregorich *et al.* (1994) considered assessment of soil organic matter as a valuable step towards identifying the overall quality of a soil. Other soil properties that should be included in a minimum data set are: soil pH (easily measured indicator) and levels of plant nutrients (total N, inorganic N, available and total P, exchangeable Ca, Mg, K). Existing data (soil surveys, soil fertility evaluation programs, long-term agronomic experiments, and reflectance spectral libraries) can be combined with newly collected data. More and more soil data are available through the internet (Motavalli *et al.*, 2002) and the number of soil studies in which existing data are used is increasing (Hartemink *et al.*, 2001). Quality verification is essential but modeling and statistical tools allow for more rigorous analysis than ever before.

IV. SOIL SAMPLING, SOIL ANALYSIS, AND ERRORS

For the monitoring of soil properties (both Types I and II data) soil chemical data are required, which are obtained by sampling and analyzing soils. Soil sampling procedures and analytical techniques have continually improved since the beginning of the twentieth century (Schuffelen, 1974; Sparks, 2003). Soil analysis is undertaken to assess the potential for a certain type of land-use, to characterize mapping units in a soil survey, and as a basis for fertilizer recommendations. Most soil analysis is undertaken for diagnosing soil constraints for agriculture. Usually the analysis is for chemical properties, and biological and physical tests are more rare (McLaughlin *et al.*, 1999). An increasing number of soil chemical measurements is undertaken to assess the risk for ecological and human health and for environmental regulations (Sparks, 1996). To a lesser extent, soil chemical analysis is undertaken for building or road construction. Three sources of errors can be distinguished: (i) during the sampling and handling of the soil samples, (ii) during the laboratory analysis, and (iii) in the interpretation of the results.

A. ERRORS IN SOIL SAMPLING

Errors in soil sampling have been well documented and are generally greater than errors in the actual soil analysis (Cline, 1944; Tan, 1996).

A key problem is that soil volumes, not areas, are sampled. Results are nearly always expressed as units per mass and not on a volume or area base, which would require measurement of bulk density. Sampling depth should be in line with the depth of soil horizons or the rooting depth of the crop. The extent that a soil sample represents the population sampled depends upon the soil variability, the number of sampling units contributing (i.e., the number of subsamples or cores), and the method of soil sampling (Cline, 1944). The problem is that soil variation is not known until soil samples have been taken and analyzed. Much progress has been made in the quantification of soil variability and how many samples should be taken to characterize soil properties (McBratney *et al.*, 2000). Soil sampling equipment is also a potential source of error when it is difficult to clean (contamination) or take cores or slices with different volumes. Also variation in sampling techniques between individual samplers may introduce errors that could be as large as 6% (Kulmatiski and Beard, 2004).

The number of subsamples or cores is an important consideration and the minimum number to cope with soil variability differs per soil chemical property, soil type, and cropping system. Based on a review of studies in Australia, Brown (1999) suggested a minimum of 5–10 cores to characterize organic C and total N; 10–20 cores for pH and exchangeable cations; 20–30 cores for extractable P, and larger numbers when inorganic fertilizer or lime is applied (Table III).

B. ERRORS IN SOIL HANDLING AND STORAGE

Soil properties may change during transport to the laboratory, but little work has been carried out on the effects of temperature, moisture, etc. on soil samples in transit from the field to the laboratory (Brown, 1999). Biological activity continues so that there can be rapid changes in soil nutrient contents.

In most cases, soils are air dried at ambient temperature and humidity in the laboratory, which affects some soil properties. Both temperature (Molloy and Lockman, 1979) and method of drying (Payne and Rechcigl, 1989) affect soil chemical properties. Tan (1996) and Landon (1991) listed the effects of air-drying based on earlier works. Air-drying will not affect total C or total N but affects $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$. The pH may be lowered by air-drying and is strongly altered in soils rich in S. Drying can cause changes in P-fixation, which is related to changes in Al and Fe chemistry. In soils with a low pH, P soluble in diluted acid tends to increase, whereas P levels tend to decrease in high pH soils. Potassium may be fixed or released from the fixed form during drying, but it depends on the type of clay minerals present. During drying exchangeable K tends to increase in soils low in exchangeable K, whereas more K becomes fixed in soils with moderate or high K content.

Table III
Soil Parameters, Their Variation and Number of Subsamples Required

Property	Vertical changes	Variation	Recommended number of samples	Random error (Landon, 1991)
pH	Often increases with depth. In strongly leached soils, acidity increases with depth	SD = 0.03–0.90; 80% of studies SD < 0.5	30–40 but 10–20 where site is uniform and lime never applied	0.2 units
Organic C	Highest near the surface sometimes accumulating as mat; may be high in subsoils of podzols and buried profiles	CV = 6–74%; 80% of studies CV < 15%	5–10, but larger number is required when surface organic matter varies	5%
Total N	Closely associated with organic C	CV = 1–85%; 80% of studies CV < 20%	As for organic C	5%
Extractable P	Almost always concentrated in the topsoil, or where fertilizer P applied in bands or drill rows	CV = 8–126%; 80% of studies CV < 90%	30–40, or 50–60 where fertilizer recently spread or banded or in paddocks with high stocking rates	10%
Exchangeable cations	Generally increases when clay content increases or where organic matter is high	CV = 3–224%; 80% of studies CV < 70%	30–40, or 10–20 where site is uniform and no amendments are applied	5%

Modified from Brown (1999) and Landon (1991).

Storage of soil samples is needed for Type I data when samples taken in the past are reanalyzed and compared to newly collected soil samples. Except for air-dried soils, samples may also be stored refrigerated but this cannot be recommended for long-term storage because of possible shifts in microbial community and the potential development of anaerobic conditions. Freezing at temperatures below -20°C can be suitable for long-term storage, given that microbial activity is effectively minimized, although it has some drawbacks. Freezing promotes desiccation, lyses microbial cells and disrupts soil organic matter structure, and it may alter exchangeable ammonium and soluble P concentrations. Typically there is a flush of biological activity in thawed soils due to the decomposition of soil microbial cells lysed by the freezing (Boone *et al.*, 1999).

Air-dried soil samples, which are not kept in airtight containers, may absorb NH_3 , SO_3 , or SO_2 gases and, therefore, the container should be made of materials that will not contaminate the soil samples (Tan, 1996). Even in airtight containers soil changes occur during prolonged storage. An important change that may occur in stored soil samples is an increase in surface acidity and increased solubility and oxidizability of soil organic matter (Bartlett and James, 1980). Research on Australian soils, however, showed that the pH (in water) of soils that were stored for 7 years was 0.55 units higher; there was no relationship between soil type and pH change due to storage of soils (Slattery and Burnett, 1992). Overall, the effects of soil storage are important for the long-term study of soil changes, but available literature on this subject is limited. There seems to be no storage condition that is perfect and the absence of a storage effect on soil properties should be checked rather than assumed (Boone *et al.*, 1999). However, in reports on long-term agricultural and ecological studies storage methods and conditions of soil samples are seldom reported.

Storage is not relevant for soil samples that were analyzed in the past and which plots were resampled. For such samples, however, it is necessary that the analytical laboratory has a consistent, systematic error (Kempthorne and Allmars, 1986), which means that it should not change over time and the error should be quantified. With analytical apparatus and personnel changes, it may be difficult to keep the error constant in a soil laboratory (Kulmatiski and Beard, 2004).

C. ERRORS IN SOIL ANALYSIS

Most soil tests have been calibrated for topsoil properties. After a series of pot trials and field trials, the quantity of nutrients measured by soil tests can be expressed in terms of deficient, adequate, or toxic for the crop considered. The quantity of nutrients extracted from the soil differs from

that accumulated by the crop. Variation in soils and soil properties versus consistency of analytical methods has been a matter of concern since the beginning of the twentieth century. International soil classification systems (FAO-Unesco, WRB, Soil Taxonomy) require that soils are analyzed by standard methods in order to compare results from different parts of the globe. This implies that some soil data are almost meaningless, like for example the CEC determined by NH_4OAc at pH 7.0 in highly weathered soils. In such very acid soils there may be a considerable portion of pH dependent charge, which results in a gross overestimation of the CEC.

The selection of an inappropriate analytical method could be termed a fundamental error and it is generally perceived that selecting the right procedure is more difficult than performing the actual analysis (Tan, 1996). Most errors arise from the fact that soil variation is insufficiently dealt with, but errors could also be made in the actual analysis. In the past decades, several international programs have been developed to check laboratory errors through exchange of soil and plant samples (e.g., LABEX, WEPAL), which has improved the accuracy of many soil analytical laboratories. There are also several handbooks on soil analytical techniques (Rayment and Higginson, 1992; Sparks, 1996; Van Ranst *et al.*, 1999), and guidelines have been developed for quality management in soil laboratories (van Reeuwijk, 1998). Although the standard set of soil testing procedures is widely used, a range of new analytical techniques have emerged that allow for rapid and accurate characterization of soil chemical properties (Sparks, 2001).

D. SOIL VARIATION

Soil varies in space (between two points) and in time (between two sampling times at the same site) and across a range of scales for both space and time. Variation in soil properties between two sampling points or sampling times can be enhanced by cultivation. Soil fertility research has dealt with variation by taking a sufficiently large number of soil samples in order to differentiate treatment effects from random variance.

1. Variation by Soil Chemical Property

Variation in soil chemical properties is affected by a range of factors including the parent material from which the soil is derived, microrelief and climate, soil fauna, litter inputs, and the effects of individual plants. In agricultural systems amendments, tillage, cropping sequences, animal dung, and manure as well as artificial drainage and irrigation cause soil variation.

The degree of variation differs per soil chemical property and some properties vary more than others, both in time and space. Table III summarizes the analysis of 44 studies on soil variation in Australia (Brown, 1999) supplemented with some general information from Landon (1991).

The data from Brown (1999) were from a large number of studies with a variety of sampling methodologies and agro-ecological conditions, which explains the large variation. Moreover, there were banded applications of inorganic fertilizers. The errors quoted by Landon (1991) show that 5–10% is common for the major soil chemical properties, but these errors cannot be directly linked to the standard deviations and coefficients of variation found by Brown (1999).

Soil chemical properties may vary from year to year, between seasons in a year, or even between days depending on weather conditions and management factors. Several studies have been carried out in an attempt to find seasonal or climatic patterns in this variation, but many studies have failed because insufficient attention was given to spatial variation or laboratory variation. In soil science, spatial variation has been given more attention than temporal variation. Fewer data sets are available to study temporal variation possibly because observations over a period of time may be affected by weather, management, and unknown factors. It has been suggested that seasonal variation on some soil properties may mask differences due to soil management. Therefore, characterization of some soil chemical properties requires more than one soil sample per year (Brown, 1999), but for most standard soil chemical properties (pH, organic C, total N, etc.) short-term temporal variation is relatively small.

The number of soil samples to characterize a soil chemical property is site-specific and affected by land-use. Prasolova *et al.* (2000) used a spatial analysis of soil chemical properties to calculate the number of samples required in two *Araucaria cunninghamii* plantations (Table IV). The calculations were based on experimental estimates of the mean differences between the means for sampling dates and variance estimates of the soil properties. The

Table IV
Sample Size Required for Estimation of the pH, Organic C, Total N, and CEC at Different Levels of Error at Two Sites Under *Araucaria cunninghamii* Plantations in Subtropical Australia

	Site 1				Site 2			
	pH	Organic C	Total N	CEC	pH	Organic C	Total N	CEC
10% error	5	29	32	19	7	35	66	15
20% error	3	9	10	7	4	11	19	6

Modified from Prasolova *et al.* (2000).

results demonstrated that there were considerable differences between the two sites in the number of samples required.

2. Variation Due to Cultivation

Natural soil variability is affected by cultivation and the cropping system. Some grain crops are sown by broadcasting over the field and usually no row effects exist, that is, localized nutrient extraction or addition. Tropical crops like maize, sugarcane, or oil palm are grown in rows, which determines the rooting pattern and extraction of water and nutrients (Hartemink, 1998c). This is further influenced by soil management like the application of inorganic fertilizers in rings around trees (oil palm), which induces spatial variability (Tinker, 1960). Soil variation under oil palm is illustrated in Table V, which depicts the pH and exchangeable K values of a Typic Paleudult in an oil palm field in Malaysia (Kee *et al.*, 1995). The oil palm was fertilized with 210 kg N and 520 kg K ha⁻¹ year⁻¹ in the form of ammonium chloride and muriate of potash, respectively. The fertilizers were applied in a ring around the palm, which caused significant acidification and an increase in the levels of exchangeable K as compared to the interrow (between two rows of palms) and frond piles (area where pruned oil palm leaves are piled up).

Field scale heterogeneity may be created when crop residues are piled up and burned creating "hot spots" or concentrations of soil fertility. Soil sampling should thus consider the spatial arrangement of the crops that might have created field scale heterogeneity in soil properties. Although the cultivation-induced variation can be taken into account when the crops are still growing, it is difficult to consider such variation when the previous crop has been slashed and a new crop is planted. For example, when oil palm fields are replanted, the hotspots created by the inorganic fertilizer applications (Table V) still affect the soil sampling results. Also old tree rooting

Table V
Field Scale Heterogeneity in pH and Exchangeable K ($n = 4$) in a 20-Year Old Oil Palm Plantation in Malaysia

Sampling depth (m)	pH (1:2.5 w/v)			Exchangeable K (mmol _c kg ⁻¹)		
	Palm circles	Interrows	Frond piles	Palm circles	Interrows	Frond piles
0-0.15	3.4	4.4	4.3	8.4	3.1	2.9
0.15-0.30	3.5	4.2	4.4	8.8	2.8	3.4
0.30-0.45	3.5	4.1	4.2	8.5	2.3	3.1

Only circles around the palm received N and K fertilizer. Modified from Kee *et al.* (1995).

Table VI
Soil Fertility Status Under Sugarcane (Within and Interrow)

	Sampling depth (m)	Sugarcane within rows	Sugarcane interrows
pH (1:5, water)	0–0.15	6.1 ± 0.3	6.2 ± 0.4
	0.15–0.30	6.4 ± 0.2	6.6 ± 0.2
Organic C (g kg ⁻¹)	0–0.15	34.1 ± 3.6	32.0 ± 2.4
	0.15–0.30	29.0 ± 2.8	22.0 ± 7.4
Total N (g kg ⁻¹)	0–0.15	2.3 ± 1.6	1.8 ± 0.3
	0.15–0.30	1.4 ± 0.2	1.2 ± 0.5
Available P (mg kg ⁻¹)	0–0.15	22 ± 10	22 ± 11
	0.15–0.30	17 ± 10	11 ± 7
Exchangeable Ca (mmol _c kg ⁻¹)	0–0.15	278 ± 73	280 ± 49
	0.15–0.30	280 ± 61	249 ± 74
Exchangeable Mg (mmol _c kg ⁻¹)	0–0.15	104 ± 16	91 ± 12
	0.15–0.30	104 ± 19	93 ± 26
Exchangeable K (mmol _c kg ⁻¹)	0–0.15	10.8 ± 4.9	10.3 ± 5.5
	0.15–0.30	6.4 ± 5.8	4.1 ± 1.8

Values are the arithmetic mean of five samples ± 1 SD. Modified from Hartemink (1998b).

patterns affect the results of soil sampling replanted fields (Dockersmith *et al.*, 1999).

As mentioned, crops grown in rows cause localized nutrient removal and create soil heterogeneity. Table VI shows soil chemical data from a sugarcane field whereby samples were taken in between the plants (in the rows) and between the rows (Hartemink, 1998b). Soil chemical properties differ between and within the rows, and to a large extent this was due to differences in rootability and soil physical factors.

V. SOIL CHEMICAL CHANGES AND NUTRIENT REMOVAL

In agro-ecosystems most nutrient output takes place by the crop removal. Different crops remove different quantities of nutrients in different ratios. Nutrient removal data by the crop are sometimes the only quantified nutrient output in nutrient balance studies.

A. ANNUAL AND PERENNIAL CROPS

There is a wide range in nutrient removal for annual crops (Table VII) and this is related to differences in cultivars, time of sampling, and agro-ecologies, which affect yield and thus nutrient removal. Variation is also the

Table VII
Nutrient Removal (kg ha⁻¹) by Annual Crops

Crop	Yield (kg ha ⁻¹)	Nutrients in kg ha ⁻¹					Reference
		N	P	K	Ca	Mg	
Maize (grain)	1000	18–77	2.2–9.7	8–72	5–14	3.3–10.7	(Boxman and Janssen, 1990)
	1100	17	3	3	0.2	1	(Cooke, 1982)
	2500	40	9	33	7.5	5.0	(Sanchez, 1976)
	12,500	298	55	247	nd	nd	(IPI, 1995)
Cassava	8000	30	10	50	20	10	(Sanchez, 1976)
	11,000	25	3	65	6	nd	(Cooke, 1982)
	45,000	202	32	286	nd	nd	(IPI, 1995)
Yam	11,000	38	3	39	0.7	nd	(Cooke, 1982)
Sweet potato	16,500	72	8	88	nd	nd	(Sanchez, 1976)
	34,500	175	34	290	nd	nd	(IPI, 1995)
Groundnut	800	30	2.2	5	1	1	(Cooke, 1982)
	1000	51–62	2.8–3.5	7–17	12–19	4.0–6.7	(Boxman and Janssen, 1990)
Soybean	1000	49	7.2	21	nd	nd	(Sanchez, 1976)
	1000	79–97	6.4–7.8	46–60	nd	4.7–5.4	(Boxman and Janssen, 1990)
	3400	210	22	60	nd	nd	(Cooke, 1982)

nd, no data.

result of different crop parts that are measured. In the literature, it is not always indicated what was included in the measurements, and husks and cobs are sometimes included whereas in other studies the nutrients in these plant parts were excluded. Also nutrients in belowground biomass other than harvested parts are seldom reported.

Nutrient removal data for some perennial crops are given in Table VIII. There are several woody perennials that are heavy K-consumers (oil palm, coffee) whereas other crops remove mostly N. Bananas, sugarcane, and sisal are also heavy K-consumers. Nutrients in the yield of perennial crops are a fraction of the nutrients immobilized in the above- and belowground biomass, as was shown for cocoa (Hartemink, 2005).

Nutrient accumulation in the belowground biomass should be considered as a transformation of nutrients—not as a loss. This applies to both annual and perennial crops although the time scale is different. At the end of a crop cycle in a perennial crop system, the trees are slashed and burned or left to decompose. The nutrients in the above- and belowground biomass are returned to the soil. During the crop cycle the nutrients have been withdrawn from the soil solution. The withdrawal is only temporary, that is, 10 years for sisal, 20–30 years for oil palm or other perennial crops grown in the tropics. Some of the nutrients taken up are recycled during the crop cycle,

Table VIII
Nutrient Removal (kg ha⁻¹) by Perennial Crops

Type	Crop	Yield (kg ha ⁻¹)	Nutrients in kg ha ⁻¹					Reference
			N	P	K	Ca	Mg	
Woody perennial crops	Oil palm	2500 (oil)	162	30	217	36	38	(Cooke, 1982)
		15,000	90	8.8	112	28	nd	(Sanchez, 1976)
		24,600	193	36	249	nd	nd	(IPI, 1995)
	Rubber	1100	7	1	4	nd	nd	(Cooke, 1982)
	Cocoa	500	10	2.2	5	1	1	(Sanchez, 1976)
		1000	19.3	4.6	10.9	1.3	3.4	(Heuvel dop <i>et al.</i> , 1988)
	Coffee	1200	24	4	36	nd	nd	(Cooke, 1982)
		2000	253	19	232	nd	nd	(IPI, 1995)
	Tea	600	31	2.3	15	2	nd	(Sanchez, 1976)
		1300	60	5	30	6	3	(Cooke, 1982)
Herbaceous perennial crops	Coconut	1400	62	17	56	6	12	(Cooke, 1982)
	Sugarcane	88,000	45	25	121	nd	nd	(Cooke, 1982)
	Bananas	45,000	78	22	224	nd	nd	(Cooke, 1982)
		30,000	85	10	226	72	90	(Sanchez, 1976)
	Pineapple	12,500	9	2.3	29	3	nd	(Sanchez, 1976)

nd, no data.

like litterfall and throughfall, which can be very high (particularly for K) in tree crop systems (Parker, 1983).

Overall, the concept of nutrient uptake, removal, and recycling is no different in perennial crop systems but important differences are the time scale or the length of the crop cycle and the much greater biomass in perennial crops.

B. NUTRIENTS IN THE ROOTS AND CROP RESIDUES

In most field crop studies, root biomass production and nutrient removal by the roots receive little attention. The reasons are obvious: the root system is hidden from direct observation and the quantification of roots is tedious and difficult because of problems in extracting roots from the soil. It is also complex because of the spatial and temporal variability of roots in the soil matrix. Despite these problems various destructive and nondestructive methods have been developed to study roots of field crops (Taylor *et al.*, 1991) in addition to sampling schemes for their quantification (van Noordwijk *et al.*, 1985). Much of the research on roots is conducted in the temperate regions and information on root biomass and its nutrient content in tropical crops is limited, with the exception of agroforestry research (Govindarajan *et al.*, 1996; Jama *et al.*, 1998; Suprayogo *et al.*, 2002).

Table IX
Nutrient Uptake ($\text{kg ha}^{-1} \pm 1 \text{ SD}$) of Sweet Potato at Two Sites in the
Humid Lowlands of Papua New Guinea

Site	Plant part	Fresh yield (Mg ha^{-1})	Nutrients in kg ha^{-1}				
			N	P	K	Ca	Mg
Hobu	Marketable tubers	18.2 ± 3.7	30 ± 6	12 ± 2	93 ± 20	5 ± 1	5 ± 1
	Nonmarketable tubers	4.0 ± 1.0	8 ± 2	3 ± 1	25 ± 6	1 ± 0.5	1 ± 0.5
	Vines	26.2 ± 4.8	80 ± 8	18 ± 2	180 ± 30	61 ± 13	20 ± 2
	Total		118 ± 10	33 ± 3	298 ± 46	67 ± 12	26 ± 2
Unitech	Marketable tubers	9.0 ± 3.8	15 ± 17	7 ± 3	39 ± 19	4 ± 2	2 ± 1
	Nonmarketable tubers	2.9 ± 1.3	5 ± 5	2 ± 1	12 ± 5	1 ± 0.5	1 ± 0.5
	Vines	30.1 ± 8.2	590 ± 21	22 ± 2	189 ± 15	37 ± 8	10 ± 2
	Total		79 ± 40	31 ± 5	241 ± 23	42 ± 10	13 ± 3

Hobu soils were classified as Typic Eutropepts and the soils at Unitech were Typic Tropofluvents (Hartemink *et al.*, 2000).

In annual crops only part of the total amount of nutrients taken up is removed by the economic produce viz. the grain of wheat, the tubers of sweet potato, or the seeds of soybean. An important portion of the nutrients taken up may be returned to the soil with the cropping residues. Table IX gives the total nutrient uptake of sweet potato tubers and vines (= above-ground biomass); less than one-third is found in the marketable tubers (= economic produce). Farmers only remove the tubers from the field and the vines remain behind as crop residues. As vines decompose, nutrients become available for the subsequent crop. Less than 25% of the total N and K uptake was found in the economic produce. It is generally recognized that crop residues are extremely important for recycling of nutrients in many cropping systems in the tropics (Giller *et al.*, 1997; Kumar and Goh, 2000).

VI. PRESENTATIONS OF RESULTS

For Type I data (monitoring soil properties over time) it is essential that the methods of soil analysis have not changed, that is, comparing soil organic C determined by the Walkley & Black method in 1970 to values obtained from the same field using a dry-combustion analyzer in the year 2000 is less than ideal. Provided analytical methods are unchanged, simple *t*-tests or analyses of variance can be used to detect statistically significant differences. An example of Type I data are given in Table X, whereas Type II

Table X
Topsoil Chemical Properties of Fluvents and Vertisols Between 1979 and 1996 (Arithmetic Mean \pm 1 SD) of a Sugarcane Plantation in Papua New Guinea

	Year	Number of samples	pH 1:2.5, water	Organic C (g kg ⁻¹)	Available P (mg kg ⁻¹)	CEC pH 7 (mmol _c kg ⁻¹)	Exchangeable cations (mmol _c kg ⁻¹)			Base saturation (%)
							Ca	Mg	K	
Fluvents	1979	15	6.5 \pm 0.4	58 \pm 15	nd	389 \pm 43	228 \pm 78	93 \pm 41	13.0 \pm 5.0	79 \pm 17
	1982	14	6.2 \pm 0.1	nd	36 \pm 4	459 \pm 55	275 \pm 35	113 \pm 24	12.9 \pm 2.0	87 \pm 2
	1983	44	6.3 \pm 0.1	nd	37 \pm 10	435 \pm 48	256 \pm 35	100 \pm 16	12.4 \pm 2.8	85 \pm 3
	1984	9	6.1 \pm 0.1	nd	42 \pm 10	437 \pm 52	266 \pm 45	102 \pm 21	12.9 \pm 3.8	87 \pm 4
	1994	12	5.9 \pm 0.1	35 \pm 6	28 \pm 9	384 \pm 65	232 \pm 47	101 \pm 22	10.8 \pm 2.3	90 \pm 5
	1996	8	5.8 \pm 0.2	31 \pm 7	28 \pm 12	374 \pm 33	220 \pm 30	99 \pm 13	8.0 \pm 2.0	88 \pm 8
Vertisols	1979	6	6.6 \pm 0.1	52 \pm 9	nd	421 \pm 21	293 \pm 69	123 \pm 39	15.5 \pm 2.7	93 \pm 17
	1982	17	6.2 \pm 0.1	nd	43 \pm 5	490 \pm 29	286 \pm 22	131 \pm 16	16.1 \pm 2.9	89 \pm 2
	1983	40	6.3 \pm 0.2	nd	40 \pm 13	477 \pm 94	290 \pm 83	114 \pm 33	12.9 \pm 2.3	87 \pm 9
	1986	7	6.2 \pm 0.2	nd	37 \pm 18	490 \pm 108	307 \pm 77	112 \pm 37	12.3 \pm 5.6	88 \pm 3
	1994	12	5.9 \pm 0.1	32 \pm 3	32 \pm 11	452 \pm 79	273 \pm 50	129 \pm 34	13.4 \pm 3.9	92 \pm 5
	1996	12	5.8 \pm 0.2	32 \pm 6	28 \pm 11	421 \pm 102	276 \pm 73	115 \pm 38	9.0 \pm 3.0	92 \pm 8

nd, no data.
Type I data modified from Hartemink (1998c).

Table XI
Soil Analytical Data of Under Bush Vegetation and Permanent Cropping in Northeast Tanzania

Land-use system ^a	Oxisols		Ultisols		Psamments		Inceptisols on limestone	
	Bush vegetation	Permanent cropping	Bush vegetation	Permanent cropping	Bush vegetation	Permanent cropping	Bush vegetation	Permanent cropping
pH 1:2.5, water	6.2	5.2	6.1	4.6	6.3	5.3	7.5	7.4
Organic C (g kg ⁻¹)	21	17	15	11	7	7	19	34
Available P (mg kg ⁻¹)	3	3	3	<0.5	3	2	9	4
CEC (NH ₄ OAc pH 7) (mmol _c kg ⁻¹)	125	88	157	110	98	60	310	310
Exchangeable Ca (mmol _c kg ⁻¹)	68	13	38	11	27	12	161	140
Exchangeable Mg (mmol _c kg ⁻¹)	26	5	23	5	14	4	70	36
Exchangeable K (mmol _c kg ⁻¹)	5	1	5	3	3	2	3	1
Base saturation (%)	80	21	45	24	47	28	76	58
Exchangeable Al (mmol _c kg ⁻¹)	0	9	0	nd	0	0	0	0
Al saturation (% ECEC ^c)	0	32	0	–	0	0	0	0

Type II data modified from Hartemink (1997b).

^aSampled sites were within 100 m distance.

^bAluminium saturation of the ECEC is calculated as: (Al/Ca + Mg + K + Na + H + Al) * 100.

nd, no data.

data are given in Table XI. Both these comparisons allow for conclusions on the effects of continuous cultivation on soil chemical properties (Hartemink, 2003). There are other methods to use soil chemical data to assess soil fertility decline including calculations on the rates of change and using paired sequential samples—these methods are discussed in a later section.

A. RATES OF CHANGE

For each soil chemical property (χ) measured over a given time span (t), the following can be calculated:

the absolute difference: $\chi_1 - \chi_2$,

the change per year: $(\chi_1 - \chi_2)/(t_1 - t_2)$,

and the rates of change in soil chemical properties: $[(\chi_1 - \chi_2)/\chi_1]/(t_1 - t_2) \times 100$,

which gives the change in percentage per year of the initial level t_1 .

Very few studies have been conducted in which rates of change in soil chemical properties were calculated. Calculating the rate of change in percentage per year using two data points assumes a linear change in a soil property. However, many soil chemical processes are nonlinear and the rate of change therefore differs at different periods (Jenny, 1980). For example, an average decline in organic C at a rate of $-0.3 \text{ g kg}^{-1} \text{ year}^{-1}$ observed between 1980 and 2000 may have been $-0.8 \text{ g C kg}^{-1} \text{ year}^{-1}$ in the 1980s, but less than $-0.2 \text{ g C kg}^{-1} \text{ year}^{-1}$ in the 1990s. This is further discussed in Section VII.

A different method is to assume that loss of a nutrient, χ , is a first order kinetic process that can be fitted to single exponential model. The first order process is:

$$d\chi/dt = k\chi,$$

whereby the rate factor k can be calculated from plotting $\ln\chi/\chi_0$ versus t whereby k represents the slope of the line. Calculating the k -factor gives insight in the rates of change in a property. This was suggested by Nye and Greenland (1960) and first order kinetics have been widely used in crop residue and organic matter decomposition studies. First order kinetics were used by Arnason *et al.* (1982) in a study of soil fertility decline in Belize: Table XII lists the results and shows the k -factor and the relative change in soil chemical properties of Rendols under permanent cropping in Belize. For the use of the single exponential model, several data points and relatively short time-steps are needed. This method cannot be used when soil fertility studies have only single time steps (t_1 and t_2). First order kinetics fit well for C and N but less well for exchangeable cations or pH. Overall this method provides necessary input for scenario studies on how soil fertility changes over time.

Table XII
Decline of Soil Fertility in Relative Values (Percentage per Year) and Calculated k -factor
Based on First Order Kinetics

Soil chemical property	Relative rate of decline (% per year)	k -factor (per year)
pH	1.2	0.013
Available P	10	0.11
Total N	4.8	0.05
Exchangeable Ca	16	0.19
Exchangeable K	3.9	0.035

Modified from Arnason *et al.* (1982).

B. PAIRED SEQUENTIAL SAMPLES

In some studies, several paired samples are available but all of them with different single time steps. This is the case when various fields are being sampled at different times, for example, some fields may have been sampled in 1987 and again in 2003 whereas other were sampled in 1992 and again in 2000. The data set from such a sampling scheme has several values of a soil property with different time steps. It is possible to calculate from such data the rate of change whereby the difference in years between the initial sample (t_1) and the second sample at (t_2), is plotted against the difference in the measured soil property values. Based on a large number of sample pairs, the decline in a soil chemical property can be calculated whereby t_1 is the initial value and t_2 the value of the second sampling. Thus, it can be calculated whether a soil property had increased or not changed (i.e., value at t_2 minus value at $t_1 \geq 0$) or whether there has been a decline (i.e., value at t_2 minus value at $t_1 < 0$).

From a sugarcane plantation in Papua New Guinea, pH data were available from 80 fields sampled at different sampling times. The difference in years between the initial sample at t_1 and the second sample at t_2 , was plotted against the difference in the measured pH values. It appeared that the decline in pH was related to the initial pH value (Fig. 1). Although the data are scattered, a larger decline occurred when the initial pH was high. This relation does not take into account the time elapsed between the pH measurements. Based on the 80 sample pairs, the decline in pH with time was calculated whereby t_1 was the initial value and t_2 the pH value of the second sampling. In only a few samples the pH_w increased or had not changed (i.e., pH at t_2 minus pH at $t_1 \geq 0$) but in the majority of the sample pairs there was a decline in pH (i.e., pH at t_2 minus pH at $t_1 < 0$). The largest decrease in pH

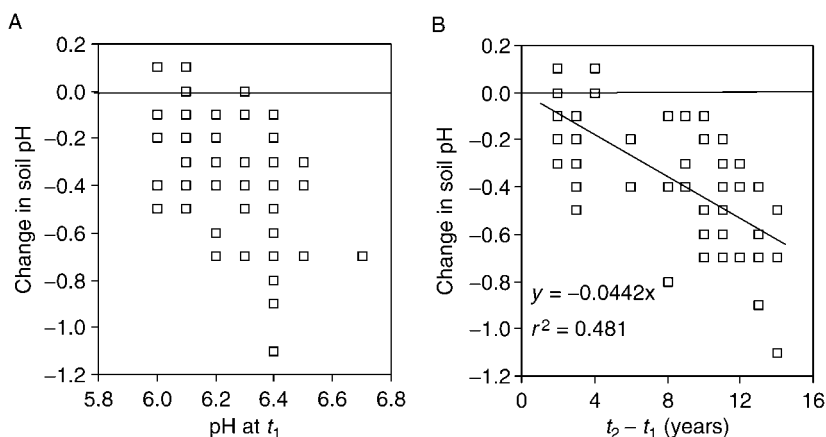


Figure 1 Changes in topsoil pH (0–0.15 m) in relation to the initial pH at t_1 (A), and the change in topsoil pH with time (B). Based on 80 sample pairs. Type I data. Modified from Hartemink (1998a).

occurred after 10 years ($t_2 - t_1 \geq 10$), and nearly 50% of the variation was explained by the linear function:

$$\Delta\text{pH} = -0.444 \times (t_2 - t_1).$$

This method proved useful to quantify rates of change in a soil property using paired sequential sample data with different time steps.

C. BULK DENSITY

Cropping brings about changes in soil physical and soil biological properties and these also influence soil chemical properties. For example, changes in the soil moisture or temperature regime affect soil microbial biomass, which influences mineralization of organic matter and other processes. Measured changes in soil chemical properties are a net effect of these processes but such changes also depend on the bulk density of the soil, which may alter under cropping. In the previous section, changes in soil chemical properties were mostly expressed as concentrations, for example, $\text{mmol}_c \text{kg}^{-1}$ or g kg^{-1} . Nutrient concentration can be expressed as nutrient content (kg ha^{-1}), which can be used in nutrient balance studies and translated in nutrient replacement by inorganic fertilizers or other amendments.

Suppose an Alfisol cropped with millet contained 1.5 g N kg^{-1} in the topsoils (0–0.20 m) in 1990, and 1.2 g N kg^{-1} in 2000. The rate of change in total N content is $0.03 \text{ g N kg}^{-1} \text{ year}^{-1}$. If the topsoil has a constant bulk density of 1.3 Mg m^{-3} , the decrease of $0.03 \text{ g N kg}^{-1} \text{ year}^{-1}$ is equivalent to

a loss of $78 \text{ kg N ha}^{-1} \text{ year}^{-1}$. This figure is easy to deal with, particularly when it is expressed as inorganic fertilizer: the loss of N from the topsoil is equivalent to 170 kg urea or 390 kg sulphate of ammonia. A further step is to translate this nutrient loss into economic terms (Alfsen *et al.*, 1997; Drechsel and Gyiele, 1999; FAO, 2001a).

As this example showed, expressing soil chemical properties in kg nutrient ha^{-1} requires soil bulk density values, which are rarely measured in soil fertility studies. Moreover, many soil chemical properties are determined by an extraction method and the values are expressed in terms of availability. Available means that the nutrient is susceptible to absorption by plants, whereas availability means effective quantity (Black, 1993). The amount of available nutrients extracted may hold little relation with the total amount of the nutrient in the soil and its availability over a given time span. The availability aspect is irrelevant for C and N because total pools are measured. Bulk density measurements thus improve the quantification of C and N loss as it would be possible to relate N loss to the total N pools. For P, K, or Ca that is not possible unless the total element concentrations were determined.

D. BULK DENSITY EFFECTS ON NUTRIENT STOCKS

Bulk density is likely to change under cropping, which has several effects. In annual cropping systems where no mechanization is used, increases in bulk density are not so likely to occur. Increases may be caused by people in the field or occur naturally; generally these increases are not spectacular. In mechanized annual cropping systems, where tractor traffic is common, substantial compaction may occur (Soane, 1990), which may affect the outcome of nutrient stock calculations. It may severely reduce nutrient availability (Arvidsson, 1999; Lipiec and Stepniewski, 1995) because rooting is restricted which limits the volume of soil from which nutrients can be extracted. In compacted soils the crop also becomes susceptible to water stress, which may have a larger impact than the reduced nutrient availability. It is difficult to distinguish these factors and their effects on crop productivity. When the soil is more compacted the thickness of the layer decreases. This means that, if the sampling depth remains the same, part of the subsoil is being sampled, which affects calculations on nutrient contents. So sampling should be corrected for decrease in the thickness of the compacted layer (Dias and Nortcliff, 1985).

An increase in bulk density does not mean that nutrient content is reduced. Table XIII shows the nutrient concentration and nutrient content of an Oxisol cropped with sugarcane. The nutrient content was calculated for three depths using bulk densities determined in 1978 and 1983. Absolute and relative differences in the nutrient concentration and nutrient content

Table XIII
Nutrient Concentration and Nutrient Content of Oxisols Under Sugarcane in 1978 and in 1983

Sampling depth (m)	Soil property	Nutrient concentration		Difference		Nutrient content (kg ha ⁻¹)		Difference	
		1978	1983	Absolute	Percentage	1978	1983	Absolute	Percentage
0-0.12	BD (Mg m ⁻³)	0.76	1.02	+0.26	+34				
	Organic C (g kg ⁻¹)	68.2	41.3	-26.9	-39	62,198	50,551	-11647	-19
	Total N (g kg ⁻¹)	4.0	1.9	-2.1	-53	3,648	2,326	-1322	-36
	Total P (g kg ⁻¹)	1.1	0.9	-0.02	-18	1,003	1,102	+99	+10
	Ca (mmol _c kg ⁻¹)	29.0	9.1	-19.9	-69	530	223	-307	-58
	Mg (mmol _c kg ⁻¹)	2.9	1.6	-1.3	-45	32	24	-8	-26
0.30-0.40	K (mmol _c kg ⁻¹)	3.0	1.2	-1.8	-60	107	57	-50	-46
	BD (Mg m ⁻³)	0.86	1.06	+0.20	+23				
	Organic C (g kg ⁻¹)	7.1	10.9	+3.8	+54	6,106	11,554	+5448	+89
	Total N (g kg ⁻¹)	1.2	1.0	-0.2	-17	1,032	1,060	+28	+3
	Total P (g kg ⁻¹)	0.9	1.1	+0.02	+22	774	1,166	+392	+51
	Ca (mmol _c kg ⁻¹)	1.6	4.0	+2.4	+150	28	85	+57	+208
0.70-0.80	Mg (mmol _c kg ⁻¹)	0.5	0.4	-0.1	-20	5	5	0	0
	K (mmol _c kg ⁻¹)	0.6	0.5	-0.1	-17	20	21	+1	+3
	BD (Mg m ⁻³)	1.01	1.10	+0.09	+9				
	Organic C (g kg ⁻¹)	3.6	3.0	-0.6	-17	3,636	3,300	-336	-9
	Total N (g kg ⁻¹)	0.5	0.5	0	0	505	550	+45	+9
	Total P (g kg ⁻¹)	1.1	1.1	0	0	1,111	1,210	+99	+9
	Ca (mmol _c kg ⁻¹)	2.0	2.2	+0.2	+10	40	48	+8	+20
	Mg (mmol _c kg ⁻¹)	0.4	0.2	-0.2	-50	5	3	-2	-46
	K (mmol _c kg ⁻¹)	0.3	0.5	+0.2	+67	12	22	+10	+82

Calculated from data in Masilaca *et al.* (1985).

were calculated for both periods. No correction was made for the decrease of soil layer thickness due to the increased bulk density. In the topsoils bulk density increased from 0.76 to 1.02 Mg m⁻³ between 1978 and 1983. There was a relatively lower decrease in nutrient content than in nutrient concentration. As a result of the increase in bulk density, different conclusions would be reached with regard to total P in the topsoils: total P decreased from 1.1 to 0.9 g kg⁻¹ whereas the P content of the topsoil increased by 99 kg ha⁻¹ due to the 34% increase in topsoil bulk density. Similar discrepancies can be found in some other soil chemical properties. Annual losses of total N from the topsoil exceed 200 kg ha⁻¹ but a slight increase in N contents of the subsoils was found, which may have been caused by leaching.

A second example on the effects of bulk density on soil nutrient contents is from Nigeria where Aina (1979) sampled Alfisols that had been cropped for 10 years and Alfisols that had been fallowed for 20–25 years (Table XIV). Nutrient concentration and content drastically decrease in permanently cropped soils, but the relative decrease in nutrient contents was lower. The relative decrease in soil nutrient contents is lower than the decrease in nutrient concentration with the exception of NO₃-N, which varies greatly with time.

VII. INTERPRETATION OF RESULTS

The interpretation of soil chemical data for the assessment of soil fertility decline is complex and particular to each situation. Factors affecting the interpretations are the agro-ecological conditions, the spatial and temporal boundaries of the study, the type of data, and how they were collected. Soil fertility decline must be differently appraised for soils in different agro-ecologies, but some common rules apply and these are discussed here.

A. RESILIENCE AND REVERSIBILITY

Resilience is the ability of the soil to recover from a period of stress—as for example, the cultivation of agricultural crops (Greenland and Szabolcs, 1994; Lal, 1997). Some soils withstand cultivation and quickly recover after a period of cultivation whereas others lack such capacity. This resilience is an intrinsic property of the soil. Therefore, different soils require different appraisal. Also individual soil chemical properties require a different appraisal depending on the type of land-use. For example, a decrease in exchangeable K may have more effect on potato production than a similar rate of decrease in total N. Likewise, the decrease in soil organic C may have no direct yield effect but could drastically reduce the resistance of the soil to physical deterioration, or to supply N or P to the crop.

Table XIV
Nutrient Concentration and Nutrient Content (0–0.15 m depth) of Alfisols Under Fallow and 10 Years of Cropping

Soil property	Nutrient concentration		Difference		Nutrient content (kg ha ⁻¹)		Difference	
	Fallow	Cropped	Absolute	Percentage	Fallow	Cropped	Absolute	Percentage
BD (Mg m ⁻³)	1.24	1.58	+0.34	+27				
NO ₃ -N (g kg ⁻¹)	19.3	2.3	-17.0	-88	36	5	-30	-85
Available P (g kg ⁻¹)	15.4	6.0	-9.4	-61	29	14	-14	-50
Ca (mmol _c kg ⁻¹)	45.1	15.0	-30.1	-67	1681	712	-969	-58
K (mmol _c kg ⁻¹)	2.4	0.9	-1.5	-63	174	83	-91	-52

Calculated from data in Aina (1979).

The removal of nutrients in relation to the size of the nutrient pool could be considered when evaluating soil fertility decline (Janssen, 1999). Much depends not only on how the size of the pool is measured, that is, the bioavailability concept in soil fertility, but also on the bulk density. Since Liebig it has been generally assumed that input of nutrients needs to match the output in order to sustain crop production (van Noordwijk, 1999), or in other words, replace what was lost. However, the time frame at which the replacement is required is different for different soils. Inherently fertile soils might compensate for the drain of nutrients and remain productive for a considerable period of time (the resilience concept) but, at some stage, these soils require replenishment of what was removed or lost. Inherently poor soils might need nutrient replenishment before a second crop is grown and their soil fertility declines quickly when permanently cultivated. The annual nutrient balance may largely differ between years or seasons (Sheldrick *et al.*, 2003), which should be taken into account in the replenishment concept.

Another aspect that affects the interpretation of results is the degree of reversibility of a change in a soil property. A decreasing level of exchangeable K may be less of a problem than a large decrease in soil organic C. Potassium may be replaced by inorganic fertilizers, whereas a doubling of the soil organic C content to its original level is very difficult. A strongly acidified topsoil may be easy to correct by the judicious application of lime, but it may be much harder to raise the pH of a strongly acidified subsoil (Sumner and Yamada, 2002). So the reversibility is different for the various soil attributes and it is also important to consider the depth to which the soil chemical changes have occurred.

B. THE TIME-LAG EFFECT

To assess whether soil fertility decline has occurred depends on what properties are measured and the rates at which the properties change. Fairly rapidly changing properties include organic C, N, and pH, and these properties usually reach dynamic equilibrium within 100 years in undisturbed ecosystems. The second group of features changes slowly and appear to be at equilibrium mainly because their rate of change is so slow (Yaalon, 1971).

Some soil processes, once established, continue for some time despite changes in the environment and the resistance to change may be related to what has been termed "pedogenic inertia" (Bryan and Teakle, 1949; Chadwick and Chorover, 2001). An example of a lag is the soil temperature, both diurnal and annual, which invariably lags behind the atmospheric temperature wave (Yaalon, 1971). By analogy, soil fertility may continue to decline for some time even if the cause of the decline (permanent cropping

without nutrient inputs) has been removed and the soil has been left fallow. Not all soil properties would show this effect and at the same pace.

C. FREQUENCY, PERIOD, AND TIME OF OBSERVATION

The frequency of phenomena that affect soil properties is important. For example, a single and destructive soil erosion event may take place once every 10 years and could have substantial impact on the soil fertility. On the other hand, there are very gradual processes like soil acidification (Pickett, 1991). For both rare events and slow phenomena to be recorded, long-term observations are needed.

Besides the pace of soil change, another factor is the period during which the observations are made. Whether a declining trend in a soil chemical property can be quantified depends on the property itself and the period and time of observation. This is illustrated in Fig. 2, which shows the trend in a fictitious soil chemical property over time. In Fig. 2A the soil property shows some noise or short-term variation, which may have been the result of weather conditions or management factors. This could be the variation in soil pH over the years, but on a different time scale it could be the variation in a soil property during a single day following the warming of the soil, or directly after rain or inorganic fertilizer applications. Soil chemical properties show variation at different time scales, but for most of the standard soil tests, long-term variation is of greater importance than the diurnal or short-term variation. The decline of the soil property in Fig. 2A (i.e., an interpolated line) is more or less linear.

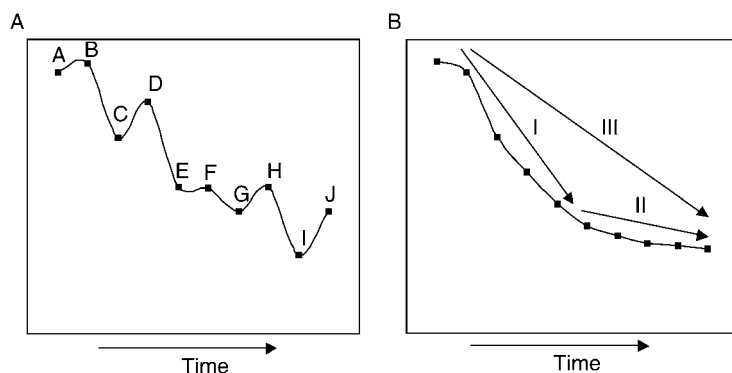


Figure 2 Theoretical changes in soil chemical properties over time when no amendments are made and the soils are permanently cropped: (A) noise and trend, and (B) exponential decline. See text for explanation (Hartemink, 2003).

A gradual decline in a soil chemical property is shown in Fig. 2B. This may represent a decline in exchangeable K in unfertilized and permanently cropped soils. Assessing of the rate of decline depends on the period and time of observation. In the beginning the decline is fast (arrow I) but, since the decline is nonlinear, the rate of decline (Δt^{-1}) is decreasing with time (arrow II). If observations would be made over time I, the rate of decline is different compared to time II even though the period of observation is the same. Rates of decline over the whole period (arrow III) would again give a different rate and this would largely ignore the nonlinearity of the relationship. It is not necessarily the case that the decline based on III is half the sum of I and II. To assess a nonlinear decline in a soil chemical property measurements at relatively short time steps are required. If time steps are large, it should be known whether period I, II, or III is evaluated.

The pattern in Fig. 2A may result in different conclusions when two points in the curves are compared. This is exemplified in Table XV where long-term, medium-term, and short-term comparisons are grouped. Comparisons were termed long-term when they exceeded five data points of the x -axis (time), medium-term when there were three to four data points, and short-term when there were two or less between two data points.

The general pattern emerging is that long-term observations yield a stronger decline in soil fertility whereas short-term observations yield no clear pattern. Due to short-term variation there is also a difference within the periods of comparison. A large decrease in the soil property was found in 20% of the long-term comparisons, whereas 70% of the comparisons yielded

Table XV
Changes in a Soil Property Between Different Sampling Times (A, B, C, D, etc.)—Based on Fig. 2A

	B	C	D	E	F	G	H	I	J	
A	+/-	-	+/-	-	-	-	-	-	-	Long-term comparison
B		+	+/-	-	-	-	-	-	-	
C			+	-	-	-	+/-	-	+/-	
D				-	-	-	-	-	-	
E					+/-	+/-	+/-	-	+/-	Medium-term comparison
F						+/-	+/-	-	+/-	
G							+/-	-	+/-	
H								-	+/-	Short-term comparison
I									+	

-- Large decrease +/- no change
 - Moderate decrease + Moderate increase
 From Hartemink (2003).

a moderate decrease (Table XV). In 10% of the long-term comparisons, no change was apparent. Medium-term comparisons yielded a large decrease in 9% of the cases, whereas in more than 25% of the comparisons no change was found. Short-term comparisons yielded no change in soil properties in almost half of the cases and a moderate increase in 15% of the comparisons.

VIII. SUMMARY AND CONCLUSIONS

In this chapter, both theoretical and practical aspects for the assessment of soil fertility decline are critically reviewed. Evaluating soil fertility decline can be addressed with different types of data. There are data from measured soil chemical properties, and such data can be from the same plot at different times (Type I data), or from plots under different land-use (Type II data). Both data types have their merits and drawbacks: data are either quickly collected and indicative of what is going on, or the collection is more tedious but the data may be easier to interpret and more meaningful.

Whatever data are collected, it is important that the boundary conditions are properly set. This means that the study should indicate whether soil fertility decline is assessed for a point, catchment, region, country, etc. At the catchment level, soil fertility may decline in one soil, but it may increase in a lower part of the catchment, which illustrates the need for the delineation of spatial boundaries. Soil fertility decline studies should also have temporal boundaries. In general, long-term observations yield better results. This review has also shown that frequency of observations is dependent on the type of study and is different for various soil chemical properties.

An important aspect in soil fertility decline studies is the spatial and temporal variation in soil properties. Soil spatial variation has been sufficiently tackled by research and various methods exist to quantify the variation. Temporal variation is a more difficult issue and fewer studies are available. As with spatial variation, it requires sufficient samples before rigorous conclusions can be drawn. Temporal variation may also be confused with other trends in the data and some soil chemical properties are more vulnerable to temporal variation than others.

Soil fertility decline studies depend on soil sampling, soil analysis, and interpretation of the results. Errors are possible in all three steps, although most errors are generally being made during soil sampling because soil variation is insufficiently dealt with and insufficient samples are taken. The choice of the analytical technique in relation to the soil property or soil type is another potential source of errors. The effects of soil sample storage and a constant laboratory error are relevant for long-term studies on soil change, but data on storage effects and laboratory errors are scarce.

Bulk density is an important factor to consider in soil fertility studies. It is needed to calculate nutrient concentrations into nutrient contents that can be used in nutrient balance studies. The decrease in the thickness of a soil layer should be considered when soils have been compacted: small deviations in bulk densities have a significant effect on the outcome of the nutrient content calculations. Nutrient removal by the economic produce is also an important component in nutrient balance studies. Published values on nutrient removal vary greatly according to differences in cultivars, measured plant portion, age of the crop, soil type, and the soil nutrient status.

For the interpretation of studies on soil fertility decline, resilience and reversibility are important concepts that reflect the ability of the soil to withstand stress and the ability to reverse changes brought about by cropping. The frequency at which observation are made also determines the interpretation of the results since some phenomena rarely occur whereas others take place gradually. The period of observation should be long enough to accommodate slow phenomena and rare events but, also, to deal with temporal variation. Due to noise in the data caused by temporal or other sources of unknown variation, different conclusion can be reached—even if the period of observation is substantial. The pattern of decline, the time of observation and the size of the time steps are important for accurately quantifying soil fertility decline.

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NEMATODE INTERACTIONS IN NATURE: MODELS FOR SUSTAINABLE CONTROL OF NEMATODE PESTS OF CROP PLANTS?

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Plant-parasitic nematodes are major crop pests in agro-ecosystems while in nature their impact may range from substantial to no significant growth reduction. The aim of this review is to determine if nematode population control in natural ecosystems may provide us with a model for enhancing sustainable control of nematodes in crops.

In agricultural systems, monocultures, narrow rotations, alteration of the soil habitat, and fertilization may alter plant-parasitic nematode dynamics and boost nematode numbers while reducing diversity and effectiveness of top-down control organisms and protective mutualisms (endophytes and arbuscular mycorrhizal fungi). Traditional agro-ecosystems (still applied in tropical regions) involve the development of complex practices such as a broad range of plant species of high genetic diversity grown in associations, rotations, and shifting cultivation, which all influence the complexity of plant-parasitic nematode communities and of control organisms.

In nature, plant-parasitic nematodes (and other root feeders and soil pathogens) drive plant community processes, such as succession and plant species diversity. Natural soils contain a wide variety of potential nematode control organisms, but the consequences of this diversity are not known. Wild plant populations also contain more genetic variability than crops, but consequences for coevolution and Red Queen processes for nematode populations have not been studied.

We conclude that integrated crop pest control may benefit from studying plant-parasitic nematode—natural antagonist interactions in natural systems, which have been coevolved for longer than crop-nematode—antagonist systems. Understanding how wild plants control their plant-parasitic nematodes may ultimately result in improving the sustainability of crop protection against plant-parasitic nematodes.

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I. INTRODUCTION

The dependency of current agriculture on pesticides for crop protection is an important obstacle for sustainable crop production and for protection of the environment. Pesticides influence the biological composition and functioning of agricultural soils, while their leaching to surface and

ground water is a threat for the provision of potable water and for the functioning of aquatic systems (Wall, 2004). Reduction of pesticide usage and pesticide impacts on the environment should, therefore, be a major goal of agronomy research. Major progress has been made by biological control research, particularly in the reduction of pesticides used against above-ground insect plagues. Strategies vary from the release of specific parasitoids in greenhouses to designing landscapes that support the survival and spread of biological control organisms (Altieri and Letourneau, 1982; Gurr *et al.*, 2003). In soil, biological control is much more hampered than above ground because of the complex nature of the soil structure and of the interactions in the soil food web (Brussaard *et al.*, 1997; De Ruiter *et al.*, 1995; Whipps, 2001).

Plant-parasitic nematodes are among the major soilborne crop pests, and their control relies on highly toxic compounds that have an impact on the whole soil community. Plant-parasitic nematodes cause an estimated annual US\$78 billion crop-yield loss worldwide, which makes effective control strategies highly essential (Sasser and Freckman, 1987). Interestingly, non-plant-parasitic nematodes are important components in the soil food web through their involvement in releasing mineral nutrients from the organic nutrient pool (Bardgett *et al.*, 1999; De Ruiter *et al.*, 1995; Wardle *et al.*, 1995) and in controlling numbers of insects in the soil (Strong, 1999). Therefore, controlling plant-parasitic nematodes may introduce a risk of also controlling nematodes that are beneficial for ecosystem processes.

In contrast with the many examples of plant-parasitic nematodes in agricultural ecosystems, relatively few studies in (semi)natural ecosystems have reported on nematode outbreaks. Plant-parasitic nematodes have been studied mainly in seminatural temperate grasslands, where their effects vary from strongly reducing primary productivity (Ingham and Detling, 1990; Stanton *et al.*, 1981) to having only minor effects (Seastedt *et al.*, 1987; Verschoor, 2002; Verschoor *et al.*, 2002). None of these plant-parasitic nematode effects alone resulted in complete die-out of vegetation, which may be partly due to the difficulty of relating the presence of species to their role in ecological interactions, which is a general problem in ecological studies (Harper, 1977).

Could we learn from population ecology of plant-parasitic nematodes and of their control in nature to improve crop protection and to enhance sustainability of agricultural production methods (Fig. 1)? Could we also learn from agronomy research to get a better understanding of the role of plant-parasitic nematodes in nature (Table I)? These are the two main questions for our review of literature on nematode control in crop systems and nematode population dynamics in natural systems. The avenues for improving biological control against plant-parasitic nematodes in crops

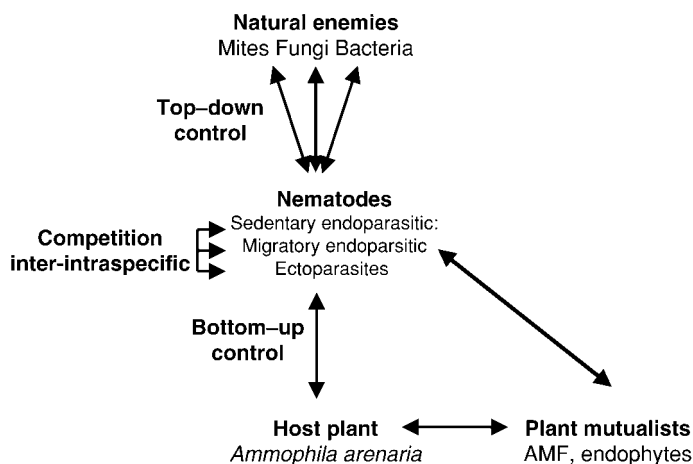


Figure 1 The players in nematode control (natural enemies, host plants, mutualists, and the nematodes themselves), the different sorts of biological control (top-down, bottom-up, and competitive interactions), and the modes of control (indicated as arrows).

and the possibilities for enhancing sustainability of agricultural production processes are discussed.

II. CURRENT PRACTICES AND OPTIONS IN NEMATODE CONTROL IN AGRICULTURE

A. CROPPING PRACTICES: INTERCROPPING AND CROP ROTATION

The presence of high densities of nematodes can be linked to a decrease in crop yield, not only by the direct effect of nematode population density but also by their interactions with other soilborne pathogens (De Ruijter and Haverkort, 1999; McSorley and Gallaher, 1993; Taylor *et al.*, 1999; Wheeler *et al.*, 1994). A number of cropping practices are used to control nematodes, sometimes unintentionally, for example, when aiming at weed control or “soil sickness” due to accumulation of soil pathogens. Crop rotation can control number of host-specific nematodes by growing non-host crops or trap plants (Fig. 2). In agricultural systems, the dynamics and distribution pattern of economically important nematodes (*Meloidogyne* spp., *Heterodera/Globodera* spp., *Pratylenchus* spp.) has been studied under different crop practices and hosts (Been and Schomaker, 2000; La Mondia, 2002; Noel and Wax,

Table I
Major Nematode Control Options Resulting from Agricultural Practices and Translation of These Control Options to Processes and Patterns in Natural Systems

Nematode control	Agriculture	Nature
Time (intraannual)	Seasonal culture	Life history variation (e.g., winter annual plants)
Time (interannual)	Crop rotation	Vegetation succession
Space (small-scale)	Intercropping (intrafield scale)	Plant community diversity
Space (large-scale)	Farm crop diversity (interfield scale)	Landscape elements: dispersal barriers; soil characteristics inundation, etc.
Genetic processes	Resistant crops breeding and genetically engineered plants	(Co)evolution, adaptation
Chemicals	Chemical control	Secondary plant compounds
Natural toxins	Green manuring	Organic matter increase (soil ripening)
Predators and antagonists	Biological control (interactions at the individual plant scale)	Multitrophic interactions (including the linking of above- and belowground interactions) around individual plants, plant competition, succession
Predators and antagonists	Suppressive soils (field scale)	Patchiness, heterogeneity within vegetation
Predators and antagonists	Integrated control (farm scale)	Ecosystem and landscape variation

2003; Nombela *et al.*, 1994; Yeates, 1994). Nematode abundance changes during the season and annual cycles differ among species and years depending on the life history of species, food availability and quality, biotic interactions with other organisms, and the physicochemical environment. The usefulness of crop rotation depends on the longevity of survival stages of the nematode.

In developing countries, the intercropping commonly used in low value crops not only controls plant-parasitic nematodes but also a range of other pests and diseases (Fig. 3). The mechanisms of control, besides starvation of the pest and disease causing organisms in the absence of suitable hosts, may be because some of the crops release nematicidal compounds such as tannins, flavonoids, glycosides (Chitwood, 2002). Crops that release nematotoxic compounds either from their roots or during decay of green plants after their incorporation in soil may be used for "biofumigation" (Jourand *et al.*, 2004a,b; Tsay *et al.*, 2004). Intercropping may also simply limit food resources for host-specific nematodes. A major limitation on the use of

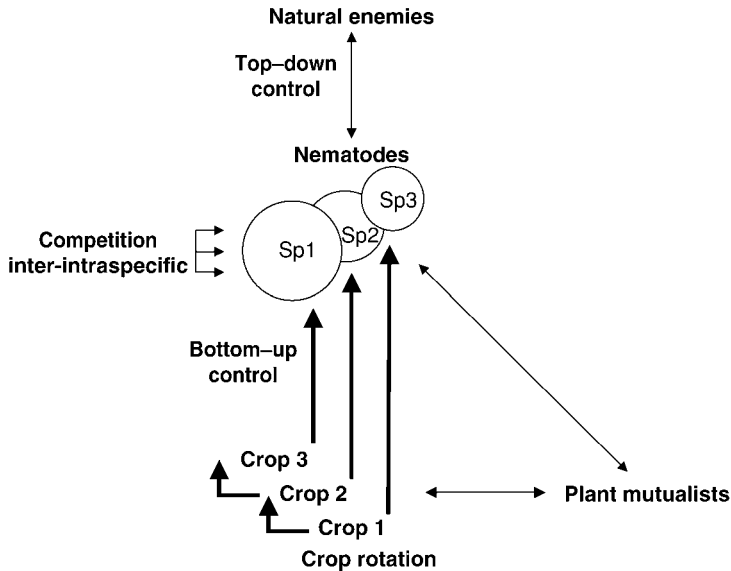


Figure 2 Influences of crop rotation on nematode control in a crop rotation system. The thickness of the arrow indicates the strength of the nematode control (the thicker the arrow, the stronger the nematodes will be controlled by the specified interaction). Crops 1, 2, and 3 are the different crops in the rotation and Sp1, Sp2, and Sp3 are the specific nematode species (this can be one species or a complex of a few nematode species) that are performing well in cultures of crops 1, 2, or 3. In all cases, symbiotic mutualists, top-down control by natural enemies and competitive interactions play a minor role in nematode control; the crop rotation itself is the most important nematode control factor.

both these cropping practices to control nematodes by disrupting the continuity of food resources is that they do not fit some intensive agricultural practices and farmers prefer to grow crops that are more economically rewarding.

B. CHEMICAL CONTROL

The most important nonspecific nematode control measure has been the use of soil fumigants such as methyl bromide, chloropicrin, 1,3 dichloropropene, metham sodium, dazomet and the use of nonfumigant, granular nematicides such as aldicarb, oxamyl, carbofuran, fenamiphos, ethoprophos, and fensulphothion (Whitehead, 1997). These nematicides are reliable and fast working, controlling populations and thereby reducing damage,

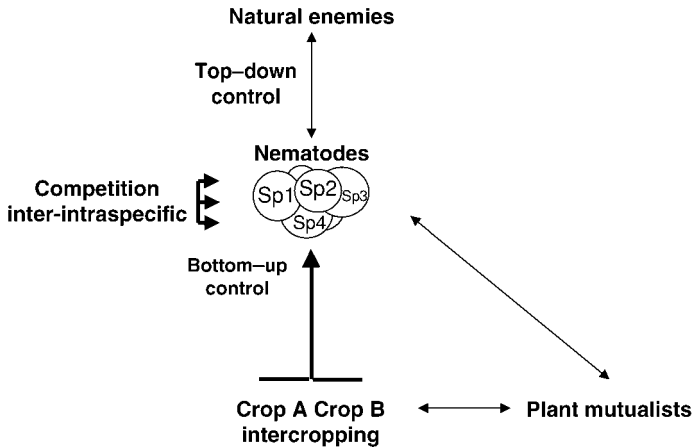


Figure 3 Influences of intercropping on nematode communities. Apart from the isolation of crop species by other crop species interspecific competition is probably the most important nematode control factor in these systems.

although not necessarily preventing posttreatment nematode reproduction and population increase. However, the use of these chemicals is increasingly restricted because inappropriate application may have negative environmental impacts and risks to human health. Moreover, side effects of these compounds may have too great an impact on nontarget organisms, including natural enemies of plant pathogens and pests and emblematic species of flora and fauna; others have direct impact as climate change forcing agents. In particular, restrictions on the use of methyl bromide and its impending withdrawal because of its ozone depleting properties have stimulated attention to the development of alternative control strategies (Schneider *et al.*, 2003).

C. BIOLOGICAL CONTROL

Usually, biocontrol strategies favor the introduction of exotic enemies (with the limitation in success due to too narrow a specificity range between the nematode and its parasite), rather than the improvement of the efficiency of indigenous enemies. The conditions of these introductions are often based on laboratory studies that focus on the binary predator–prey interaction without (or only partially) taking into account the impact of the environment on this dynamics. The interactions between soil and plant with plant-parasitic nematode life cycles and those of their enemies are inadequately understood. In tropical and subtropical countries, the ability to control

nematodes with exotic enemies remains unsatisfactory, because of their reduced adaptation to local climatic conditions or soil environments, and because of their inadequate host specificity to local pests. Consequently, studies have been performed on biocenotic mechanisms, which manage the natural development of indigenous enemies (Kerry and Hominick, 2002). The objective is to preserve or to create sustainable nonpathogenic balances, rather than to promote methodologies unable to safeguard soil biodiversity.

Organisms that affect nematodes include mites, collembola, predacious nematodes, fungi, and bacteria. Factors that impose practical limitation on the use of biological control are the difficulties in mass-producing predators and obligate parasites and the varying effectiveness in control due to interactions with soil factors, such as moisture, pH, and antagonism, by other soil organisms. Nevertheless, there are some examples of biological control developing in cropping systems. For example, the cereal cyst nematode *Heterodera avenae*, is controlled in much of Europe by fungi parasitic on females (*Nematophthora gynophila*) and eggs within cysts (*Pochonia chlamydosporia* and others). Similarly, clover cyst nematodes appear to be controlled on white clover in UK grassland by parasitic fungi; and the bacterium *Pasteuria penetrans* (or spp.) reduces numbers of root-knot nematodes (*Meloidogyne* spp.) in some cropping systems (Trudgill *et al.*, 2000). Certainly, *Pasteuria* is commonly encountered on a range of plant-parasitic and free-living nematode species in soils from natural and seminatural vegetation (Chen and Dickson, 1998).

Of the soil environmental impacts on root-knot nematode–bacteria interactions, texture, and structure are key factors (Dabiré *et al.*, 2005a; Mateille *et al.*, 2002). Root-knot nematodes, as well as their infestations by *P. penetrans* are observed more frequently in sandy than in clay soils (Bird and Brisbane, 1988; Mateille *et al.*, 1995; Spaul, 1984). But the amount of clay particles in sandy soils influences directly the ability of the soil in maintaining a pool of *P. penetrans* spores, which improves nematode infection (Dabiré and Mateille, 2004; Dabiré *et al.*, 2005b). Plant susceptibility to nematodes and its variation with cultural practices influences the proportion of *Meloidogyne* spp. juveniles infested by *P. penetrans*. The production of the bacterial spores and their concentration in soil is directly related to the development of the nematode population as *P. penetrans* is an obligate parasite. Worldwide, there is a close correlation between the vegetable species and the abundance of *Meloidogyne* spp. infested by *P. penetrans* (Giannakou and Gowen, 1996; Hewlett *et al.*, 1994; Ko *et al.*, 1995; Mateille *et al.*, 1995; Tzortzakakis *et al.*, 1995). The continuous culture of vegetables (thus greatly influencing root-knot nematodes) appears unsuitable for the production of *P. penetrans*, because the high multiplication rate of these nematodes does not allow the bacterial population to numerically keep up with host abundance. Conversely, including nonsusceptible crops in the rota-

tions allows the ratio of bacterial spores/nematodes to increase in the soil (Giannakou and Gowen, 1996).

Natural enemy communities tend to build up relatively slowly over 4–5 years and usually under perennial crops or crops grown in monocultures. This slow buildup has been demonstrated by studies on the suppression of *H. avenae* by *N. gynophila* and *P. chlamydosporia*. In such suppressive soils, about 95% of the females and eggs were destroyed by the fungal parasites (Kerry *et al.*, 1982). As well as host plant identity (De Deyn *et al.*, 2004), nematode identity may greatly influence the microbial enemy community in terms of their diversity and population dynamics (Kerry and Hominick, 2002). It has been suggested that microbial enemies have a role in the regulation of most nematode populations, but that this only becomes apparent in agricultural conditions where a suppressive soil develops (Stirling, 1991). In intensive cropping systems, a population of microbial enemies is thought to be selected from the natural enemy communities (Kerry and Hominick, 2002), but this seems to be an exception to nematode regulation by microbial enemies.

Jaffee *et al.* (1992) found that nematode parasitism by fungal antagonists (e.g., *Hirsutella rhossiliensis*) exhibited two intrinsic density-dependent qualities. The first is host threshold density, the upper limit of hosts in a given system required to prevent a pathogen from becoming extinct, and the second is temporal density-dependent parasitism. This refers to changes in nematode and antagonist density, where the latter usually lags behind increases in nematode populations. An initial enrichment of hosts (e.g., nematodes) precedes and subsequently supports the eventual buildup of beneficial organisms that lead to nematode suppression (Jaffee, 1992). This leads to a stable equilibrium between antagonist–pest populations, instead of a complete eradication of the original nematode problem (Kerry, 1977) and is the quintessential definition of biocontrol.

Symbiotic mycorrhizal fungi are widely recognized as key organisms affecting the productivity and diversity of natural plant communities (Van der Heijden *et al.*, 1998). Several mechanisms have been proposed to explain the protective role of the arbuscular mycorrhizal fungi (AMF) symbiosis (Azcón-Aguilar and Barea, 1996; Jeffries *et al.*, 2003; Little and Maun, 1996). The improved plant nutrition may compensate for nematode damage. The AMF may compete directly with the pathogens for photosynthates and infection sites or AMF reduces the suitability of the root for the pathogen through anatomical or morphological changes. AMF-infected plants may change the microbial community in the rhizosphere. There may also be associated with local elicitation of plant defense mechanisms, and/or delayed nematode development within the root tissue. The protective role of AMF against parasitic nematodes differs for ectoparasitic and endoparasitic nematodes. It seems that AMF would be more effective in either protecting the plant or increasing plant resistance against endoparasitic nematodes.

However, the effect on ectoparasitic nematodes is variable, and, in some cases a positive effect from AMF in ectoparasitic nematode populations has been found (Hol and Cook, 2005).

The obligate mutualist fungi of the genus *Neotyphodium* (formerly *Acremonium*) infect leaves and stems of grasses and in some conditions are ecologically and economically significant because of the impact of their secondary metabolites on herbivores. There is significant variation in the effectiveness of the interactions depending upon genetic and environmental factors, but some combinations of grass and fungus are fully resistant to several nematodes, including species of *Meloidogyne* and *Pratylenchus* as well as some ectoparasites (Cook and Lewis, 2001).

Studies on biological control of plant-parasitic nematodes that combine two or more microbial enemies can produce better results (Meyer and Roberts, 2002). The importance of the diversity of the natural enemy community is unknown and, in the few studies in which it has been analyzed and compared to rates of regulation of nematode populations, no correlation was found (Jaffee *et al.*, 1996, 1998; Persmark *et al.*, 1995). A wide range of natural enemies occurs in citrus orchards infested with *Tylenchulus semipenetrans* in California (Stirling and Mankau, 1977) and with *Radopholus similis* in Florida (Walter and Kaplan, 1990). The factors that retain a diverse natural enemy community or select specific species are important for our understanding of the biological control of nematodes.

Although it is difficult to generalize, nematode predators do not appear to have significant impacts on plant-parasitic nematode population densities in disturbed soils. Mites and collembola tend to be confined to the surface layers and unable to pursue nematodes into the pore spaces occupied by these prey; in arable agricultural soils, predators, including predatory nematodes are relatively scarce. Difficulties in the development of predators as biological control agents, including their lack of specificity, means that there is little detailed information on the role of predators in the dynamics of plant-parasitic nematodes. Walter *et al.* (1988) suggested that mites may be important in the regulation of nematode populations in short-grass steppe, but food web studies in soil ecosystems (De Ruiter *et al.*, 1995) and under bush lupine (Strong, 1999) suggest that predators may have little influence on plant-parasitic nematodes in natural systems.

D. ORGANIC AMENDMENTS AND SUPPRESSIVE SOILS

Manipulations of the soil environment by processes, such as the application of soil amendments, seek to stimulate the activity of soil biota that produce or improve general pest suppressiveness. The addition of organic amendments may indirectly influence nematode antagonistic fungal activity

in soil (Van den Boogert *et al.*, 1994). Rodriguez-Kabana *et al.* (1987) report that those amendments that are the most efficient at reducing nematode numbers possess low C:N ratios and have a high protein or amine-type content. Hallmann *et al.* (1999) demonstrated the suppressive effect of chitin-amended soil on *M. incognita* infestation levels. This effect was attributed solely to increases in indigenous bacterial and fungal population levels. The incorporation of plant material, such as *Tagetes minuta* synergized the antagonistic activity of the nematophagous fungus, *Paecilomyces lilacinus* toward *M. javanica* (Oduor-Owino, 2003). In general, organic amendments must be applied at high rates (>1 ton ha⁻¹) in order to have a significant effect on nematode populations and so must be cheap and locally produced for exploitation in agriculture.

A special case of biological control is a suppressive soil. In suppressive soils, diseases or nematode outbreaks do not occur (Kerry and Hominick, 2002). While it is encouraging that suppressiveness within soils has been demonstrated to occur in the field, much effort has gone into understanding the physiochemical environments and microbial community structure that underlie this phenomenon (Whipps, 1997). However, the mechanisms involved are still poorly understood (Jaffee, 1992; Oostendorp *et al.*, 1991), despite considerable research effort, this lack of an adequate understanding of the factors that influence parasitism and predation ecology in soil, has hampered the development of effective biocontrol agents (Kerry, 1987).

E. PHYSICAL CONTROL: DISTANCE AND TREATMENTS

Nematode abundance is affected by density-dependent and density-independent factors. Density dependence depends on the capacity of the population development of natural enemies to keep up with population development of the nematodes. The poor response capacity of some obligate parasites to keep up with rapid nematode population development (Kerry *et al.*, 1982) is a limitation to density-dependent nematode population control. In a long-term study (Kerry and Crump, 1998), however, nematode and fungal antagonist populations seemed to have a corresponding development over time. There are relatively few of these datasets, which limits the capacity to study consequences of density-dependent nematode control by ecological modeling.

In soil, the most common density-independent factors derive from climate and prevailing meteorological conditions, namely extremes of moisture and temperature, and from physical factors including agricultural practices such as tilling and plant destruction (Stirling, 1991). Using clean planting material, tools, and machinery are important methods of preventing nematode spread from field to field. At national and international levels, quarantine services

regulate plant transport and control, and some nematodes are the subject of these regulations. When plant-parasitic nematodes are present in cropping systems, growers adopt various strategies to limit their impact, using either methods based upon the crop itself or other approaches not specific to the crop. A modern goal is to integrate different control methods in a management scheme that tries to reduce the impact of the nematodes at farm level (rather than in individual fields) and over a consistent period (rather than just the current crop).

Control of plant-parasitic nematodes by physical treatments of soil is widespread, although not always consciously directed at nematodes and often limited by climatic and environmental conditions. Soil heating by solarization generally takes 3 weeks of bright sunshine, and may be enhanced by covering soil with plastic mulches; an advantage may be that solarization is more detrimental to plant-parasitic than to saprophytic nematodes (Ostrec and Grubisic, 2003). Induction of anaerobic conditions in soil by flooding can control root knot and other plant-parasitic nematodes (Sotomayor *et al.*, 1999) and is often done unintentionally. For instance, in China, cyst nematodes damage winter sown wheat grown in rotation with maize, but not the summer crop that is rice grown in flooded paddies. Soil disturbance by tillage can reduce nematode numbers and change the composition of the nematode community in favor of bacterial feeding nematodes (Lenz and Eisenbeis, 2000). Steam treatment of soil is not specific and is expensive, its use being limited to high value crops and for composts. Such physical impacts probably occur in natural systems but at more limited scales.

F. GENETICALLY RESISTANT CROPS

Resistance and tolerance of plants to plant-parasitic nematodes is widespread in nature. Naturally occurring resistance is discussed to include multiple genes and pathways (Caicedo and Schaal, 2004). This is in sharp contrast to their cultivated relatives, which exhibit generally a genetically simple, narrow based resistance to pathogens. This narrow resistance is the result of decades of breeding focused on, for example, high yields, fast growth, and more recently on especially vertical, that is, gene-for-gene resistance *sensu lato* (dominant R-genes). The R-genes trigger localized death of pathogen-infested cells; often referred to as a “hypersensitive plant response” (Dangl and Jones, 2001). Plant parasitic nematodes may easily break down narrow resistance, which is supposed to occur especially in local native populations, since introduced species, such as the introduction of the stem nematode *Ditylenchus dipsaci* in Australia, may have too

low genetic variation to overcome selection pressure of plant R-genes (Cook, 2004).

Although experimental work on R-genes is extensive, resistant crops are not widely used in practice (Starr *et al.*, 2002). Among resistant cultivars are those, which are genetically resistant to pathotypes of potato cyst nematodes (*Globodera rostochienis* and *G. pallida*); cereal cyst nematodes (*Heterodera avenae*); beet cyst nematodes (*H. schachtii*); root-knot nematodes (*Meloidogyne incognita*); stem nematodes (*Ditylenchus dipsaci*); and soybean nematodes (*H. glycines*) (Cook, 2004).

Besides the narrow base of resistance of cultivars, the limited use of resistant cultivar genotypes may result from the relatively high costs involved in developing resistance, such as lower yield or other unwanted side effects (Cook, 2004; Rauser, 2001). Present-day research aims at introgression of polygenic resistance (a pyramid of R-genes), instead of single dominant genes, and integrated strategies, such as rotating genotypes containing major R-genes, through time and space, that is, high dose/refuge strategy (Rauser, 2001). There are no examples of application of genetically modified crops with nematode resistance in agriculture.

In addition to genetic modes of resistance, crops may be either treated with chemicals or weak pathogens (Kempster *et al.*, 2001; Oka and Cohen, 2001; Oka *et al.*, 1999) to trigger a genetic resistance response. Both methods, however, have not been exploited in practice. Examples of application of chemicals are DL- β -amino-*n*-butyric acid (BABA) acid that reduce root-galling and egg densities of *M. javanica* (Oka *et al.*, 1999) and salicylic acid (SA) to suppress *M. incognita* (Vasyukova *et al.*, 2003) in tomato. Preventive inoculations of less aggressive or incompatible strains have shown to decrease mortality rates of hosts after attack by virulent nematodes (Kosaka *et al.*, 2001; Ogallo and McClure, 1996).

III. NEMATODES IN NATURAL SYSTEMS

All agricultural crops are derived from wild species by selection and breeding. Major selection criteria have been fast growth and high yield, but selecting genotypes with these traits may have resulted in enhanced sensitivity of the current crop species and races to pests, diseases, and climatic extremes. In the context of this review, a key question is: do nematode populations ever reach damaging population levels, or outbreak densities, in nature? Here we consider some of the ecological characteristics of the two systems in relation to how they affect nematode population dynamics.

A. VEGETATION PROCESSES: SUCCESSION, DIVERSITY, AND INVASIVENESS

Natural vegetation processes, such as succession, plant species diversity, and plant invasiveness, can be considered as natural equivalents of cropping practices. Vegetation processes can be the result of the defense strategies of wild plants against their soilborne enemies (Van der Putten, 2003). Soil pathogens (Van der Putten *et al.*, 1993) and root herbivores (Brown and Gange, 1992; De Deyn *et al.*, 2003) drive succession when early succession plant species are less tolerant to the soilborne enemies than plants that appear in later succession stages. Plant diversity is maintained mainly by negative feedbacks with the soil community (Bever, 2003). Plants that cannot control their soilborne enemies are less dominant in natural vegetation than plants that prevent fast development of soil pathogens and root parasites (Klironomos, 2002). These soilborne enemies control plant abundance (Packer and Clay, 2000), and release from soilborne enemies may enhance plant invasiveness (Callaway *et al.*, 2004; Knevel *et al.*, 2004; Reinhart *et al.*, 2003).

The role of plant-parasitic nematodes in vegetation processes has received less attention than the role of soil insects, soil pathogens, or mycorrhizal fungi. In coastal sand dunes, plant-parasitic nematodes are involved in the natural degeneration of three dominant plant species, marram grass (*Ammophila arenaria*), American beach grass (*A. breviligulata*), and sea buckthorn (*Hippophaë rhamnoides*), but their importance is not in a keystone role (De Rooij-van der Goes, 1995; Oremus and Otten, 1981; Seliskar and Huettel, 1993; Van der Stoel *et al.*, 2002; Zoon *et al.*, 1993). Invasiveness of *A. arenaria* outside Europe is correlated with less exposure to feeding-specialist plant-parasitic nematode taxa, such as root-knot and cyst nematodes (Van der Putten *et al.*, 2005), but there can still be considerable biotic resistance from the native soil community in the new habitats (Beckstead and Parker, 2003; Knevel *et al.*, 2004), also suggesting that plant-parasitic nematodes do not play a keystone role in reducing plant growth in dune systems.

In conservation grasslands, plant-parasitic nematodes were heterogeneously distributed and their impact on plant production could only be substantial in small patches with high nematode densities (Verschoor, 2002). This is in contrast with studies in prairie grass ecosystems, where plant-parasitic nematodes were considered to consume substantial amounts of net primary production [reviewed by Stanton (1988)]. This means that the role of plant-parasitic nematodes in vegetation processes is still unclear and reports on vegetation changes due to outbreaks of wild plant-parasitic nematodes are rare.

B. NEMATODE DIVERSITY, ABUNDANCE, AND DYNAMICS IN NATURE: FOOD WEB INTERACTIONS AND CONTROLS

There are a considerable number of studies analyzing the dynamics and/or the composition of plant-parasitic nematode communities of various natural and seminatural temperate ecosystems, including grasslands, coniferous forests, coastal dunes, etc. (Armendariz *et al.*, 1996; De Goede, 1993; Imaz *et al.*, 2002; Navas and Talavera, 2002; Porazinska *et al.*, 2003; Van der Stoel *et al.*, 2002; Verschoor *et al.*, 2001; Wall *et al.*, 2002). Factors that may control the diversity and abundance of plant-parasitic nematodes, however, have not been extensively studied. Plant-parasitic nematodes are very responsive to changes in vegetation (Korthals *et al.*, 2001), and plant identity greatly influences their population densities (Wardle *et al.*, 2003; Yeates, 1987). Plant species identity is also a main driver of nematode taxonomic and functional diversity, more than plant diversity (De Deyn *et al.*, 2004). Plant removal studies indicated that plant-parasitic nematodes had considerable ability to survive in the absence of host plants (Wardle *et al.*, 1999).

There seems to be more diversity in nematode communities in relatively undisturbed soil than in agricultural soils (Baujard *et al.*, 1979a,b; Cadet *et al.*, 2003a,b; Hanel, 2003; Pate *et al.*, 2000). However, there are few data on the population structures and dynamics of plant-parasitic nematodes in natural ecosystems at scales appropriate for making comparisons with crop systems. In principle, the aggregated distributions known from agriculture should also occur in natural systems, particularly for host specific nematodes. Data from some northern temperate grassland ecosystems (tabulated in De Goede and Bongers, 1998) confirms that species of *Pratylenchus* (84% incidence) are more frequently encountered than those of cyst (21%) or root-knot (19%) nematodes in plant species rich, not recently cultivated, grassland soils (>700 sample points from 13 grasslands). Population densities in these situations were on average low in comparison to the economic thresholds of comparable crop pests but occasional samples with more individuals are encountered in seminatural grasslands (Verschoor *et al.*, 2001, 2002).

Herbivores may be controlled by resources as well as by predators (Olf *et al.*, 1999). Mycorrhizal fungi (Hol and Cook, 2005) and interspecific competition (see for a review Eisenback, 1993) have been considered as possible nematode control in agriculture. However, there is very little information on the role of mycorrhizal fungi and other microbial antagonists in controlling plant-parasitic nematodes in nature. Most of these studies have concerned the control of bacterial or fungal feeding and entomopathogenic nematodes (Jaffee, 1996; Koppenhofer *et al.*, 1997). In natural dunes, plant-parasitic nematode influences on host plants can be counteracted by arbuscular mycorrhizal fungi

(De la Peña *et al.*, 2006; Little and Maun, 1996). Agricultural intensification disrupts the internal regulation of soil communities (Giller *et al.*, 1997), for example, by reducing the hyphal connections of mycorrhizal fungi (Helgason *et al.*, 1998), which limits mycorrhizal control of nematodes in intensive agriculture. Moreover, mycorrhizal infections are discouraged at high soil (especially phosphorus) fertility, so that in intensive agriculture, both physical and chemical soil conditions do not favor plant protection by mycorrhizal fungi. In sand dunes, the temporal and spatial dynamics of root-knot nematodes is limited by cyst and root-lesion nematodes (Brinkman *et al.*, 2005). In agriculture, nematode communities are dominated by only a few genera of plant-parasitic nematodes and bacterial feeders (Freckman and Ettema, 1993), which may affect the role of competitive interactions in nematode control in agro-ecosystems.

C. FROM RESISTANCE GENES TO RED QUEEN PROCESSES

The host ranges of the root-knot nematodes indicate that crop plants are much more susceptible than wild plant species (Ehwaeti *et al.*, 1999). It is clear that resistance genes to root-knot nematodes are available within species of wild or ancestral relatives of crop plants, and it is likely that gene-for-gene interactions occur(red) between the putative parental nematode species and the crop plant ancestors (Cook, 2004). In both cyst and root-knot nematodes, the evidence suggests that the gene-for-gene interactions that we seek to exploit have coevolved in situations very different from those in modern agriculture. It is of interest to consider the natural arena and see if there are lessons for enhancing the usefulness, for example, durability in the face of intraspecific variation (virulence) and nematode species selection. If the major pest species of root-knot nematodes are of hybrid origin and have been selected during crop domestication and agricultural simplification, it is not likely that we shall find effective resistance in recent ancestral crop plants. It is more likely that resistance genes will be in related wild progenitors that may have coevolved with the ancestral nematode species. If the ancestral sexual nematodes in natural systems had more restricted host ranges, it is unlikely that plants will have the full range of genes for resistance effectiveness against the polyploid parthenogenetic root-knot nematodes.

Within species, there are no data on the genetic structure of plant-parasitic nematodes in natural systems. There are examples available from animal or entomopathogenic nematodes (Blouin *et al.*, 1995, 1999; Hawdon *et al.*, 2001). For plant-parasitic nematodes, cyst nematodes have been studied at the genetic level in crops (Castagnone-Sereno *et al.*, 1993; Folkertsma *et al.*, 1996, 2001; Hugall *et al.*, 1994; Lasserre *et al.*, 1996; Navas *et al.*, 2001). The cyst nematodes show extensive levels of gene flow (Folkertsma *et al.*, 1996;

Picard *et al.*, 2004; Plantard and Porte, 2004), probably by passive dispersal of cysts via air or human vectors. This extensive gene flow would explain the relatively high genetic variability of populations, which might enhance the potential to breakdown local host plant resistance.

The ability of a nematode species to parasitize a host is measured by reproduction. Resistant hosts do not permit female nematodes to develop to reproductive maturity, and host resistance is often expressed as a hypersensitive response. Currently, these relationships appear to conform to the gene-for-gene hypothesis. Nematode populations can be classed by their different degrees of aggressiveness, defined as the relative level of reproduction that occurs on a given host genotype. Therefore, genetic analysis has been applied to understand the bases of parasitism, and unambiguous pathotype classifications have been developed for crop pests (Dong and Opperman, 1997; Semblat *et al.*, 1998, 2000; Wang *et al.*, 2001).

At the intraspecific level, there are very substantial differences between crop plant genotypes in their host status to particular nematodes. Naturally occurring genetic resistance has been used to control nematodes of some crops. The extent of genetic variation in host and nematode populations are very important determinants of durable resistance. Single dominant resistant genes in pure line crops are likely to provide durable control only in unusual situations. Human impacts on plant genetic variation during the relatively recent domestication of crops and their current use in intensive farming have also affected nematode variation. Moreover, the time frame, spatial scales and likely nature of plant-nematode coevolution in the longer period preceding domestication further emphasize the significance and extent of the genetic complexity of the interactions. It is important to take these factors into account during the identification of resistance sources and their exploitation through plant breeding.

In natural populations, genetic variation is probably critical for enabling hosts to persist; the evolutionary arms race between hosts and parasites requires genetic variation and drives the need for sexual reproduction. This is known as the Red Queen hypothesis (Clay and Kover, 1996). Red Queen processes have not yet been tested for plant-parasitic nematodes. However, plant-parasitic nematodes are not only parasites, they are also subject to predation and parasitism by a diverse natural enemy community. The nematophagous and antagonistic bacteria and fungi that have been isolated contain much variability within individual species (Kerry and Hominick, 2002). The importance of this variation in the regulation of nematode populations is unknown and it would be a major challenge to study Red Queen processes in relation to more complex food web processes.

In coevolutionary host-parasite systems, each species constitutes an ever-changing environment to which its opponent has to adapt. Traditionally, plant-pathogen populations have been considered homogenous entities in

which spatial considerations played little role. However, this situation has been proved to be too simplistic and geographic structure is now considered as an important factor in coevolutionary processes (Thompson, 1999). Different populations may show markedly diverse patterns of distribution of genetic variation for resistance and virulence. Consequently, the classical model for gene-for-gene coevolution may be expressed in different ways (Burdon and Thrall, 1999), with short-term interactions in human dominated and homogeneous agricultural systems but natural host-pathogen associations showing dynamic characteristic of metapopulations of a series of populations that show varying degrees of connectedness, with local extinction and colonization events. More knowledge of the spatial pattern of nematode distributions in natural systems may elucidate if outbreaks are significant in these systems, if we are able to define the appropriate spatial, temporal, and genetic scales of investigation (Ettema and Wardle, 2002).

D. ORIGIN OF PLANT-PARASITIC NEMATODES; IMPACT OF AGRICULTURE AND INTENSIFICATION PROCESSES

Some of the major pest species of nematodes are known to occur in natural or seminatural vegetation. This is particularly the case with cyst nematodes; those that are pests of potato (*Globodera* spp.) and soybean (*H. glycines*) are known from wild plants in the centers of diversity of the ancestors of their crop host species. Cereal cyst nematode species also occur in grasslands. The major root-knot nematode pests (*M. arenaria*, *M. incognita*, and *M. javanica*) are not known from natural ecosystems. The several hybrid origins postulated by Trudgill and Blok (2001) for *M. arenaria*, *M. incognita*, and *M. javanica* could have occurred between few ancestral species followed by polyploidy that retained genetic diversity. Hybridization may stimulate invasiveness, particularly where genetic or reproductive mechanisms fix hybridization (Ellstrand and Schierenbeck, 2000). The spread of these root-knot nematodes worldwide may therefore have occurred recently from a geological point of view, certainly in the last 10,000 years of agriculture and more probably in the last few hundred years of mass movement of colonizing people and intensification of agriculture (Cook, 2004).

From published observations, it is impossible to determine the spread of root-knot nematodes as distinct from the spread of knowledge about them. Thus, the first records of root-knot nematodes are from the United Kingdom on cucumber in 1855 and probably the first damage related observations on coffee in Brazil in 1878 and crops in Florida in 1889. Cucumbers have been cultivated for 3000 years since domestication probably in India and were grown under glass in the United Kingdom during the 18th and 19th centuries. Coffee (*Coffea arabica*) having evolved in southwestern Ethiopia

was dispersed via Arabia and India, reaching Indonesia during the late 17th century and then via Europe to the Caribbean and South America in about 1725. Production rapidly increased in Brazil after the railways were developed in the 1860s.

Many other species of both cyst and root-knot nematodes have been encountered in natural vegetation, but are usually not prominent. In the case of the foredune grass *A. arenaria*, which occurs as a natural monoculture on mobile dunes, cyst and root-knot nematodes are an important component of the nematode community (Van der Putten *et al.*, 2005). Root-knot nematodes are potentially harmful to *A. arenaria*, but interspecific competition with cyst and root-lesion nematodes (*Pratylenchus* spp.) reduces their effects on plants (Brinkman *et al.*, 2005). Orion (1979) observed that root knot is also not a problem on primitive farms in Israel, becoming one only where agriculture is improved. It has not yet been resolved whether the “improvement” effect of agriculture may act by reducing nematode diversity, by altering the genetic composition of root-knot nematode populations, or by a combination of factors, also including monocultures, intensification, irrigation, and fertilization. Shifts in composition and structure within plant-parasitic nematode communities have been observed between native and modified grasslands in New Zealand (Bell *et al.*, 2004): in modified grasslands (plowing, soil amendments, introduced species of pastures), *Heterodera* sp. and *Meloidogyne* sp. take over from the criconematids originally present in native lands. It is quite likely that the cyst and root-knot nematodes are also introduced into New Zealand. These effects may all lead to simpler cropping systems allowing maximum nematode population increase due to parasitic adaptations.

The apparently low population densities of host-specific nematodes in natural vegetation probably reflect the relatively low abundance of host plants. There has been increasing research on the role of space in population, community, and ecosystem processes in soil systems (Ettema and Wardle, 2002). The distributions of soil biota as well as the different factors that determine them, influence spatial patterns of decomposition, nutrient supply, and root herbivory, ultimately influencing the spatial structure of plant communities.

IV. LESSONS FROM LOOKING ACROSS THE FENCE

A. THEORY-DRIVEN RESEARCH APPROACH

The prime interest of applied nematological research has been focused on sampling and identification of nematodes, their physiology, molecular ecology, and virulence and interactions with their hosts, rather than on

theory development. While nematode interactions have received considerable attention in nematology (Khan, 1993), theory on multitrophic interactions has been primarily developed by entomologists (Price *et al.*, 1980). Probably a major reason for this is the complexity of the experiments needed when studying belowground multitrophic interactions (Van der Putten *et al.*, 2001). However, nematode control might benefit from a more integrated approach of plant defense, comparing top-down versus bottom-up effects (Walker and Jones, 2001), constitutive versus induced defense (Karban and Baldwin, 1997), and direct versus indirect defense (Price *et al.*, 1980).

Tolerance versus resistance has received considerable attention in nematology (Trudgill *et al.*, 2000) and specialism versus generalism may probably be quite different from what is known of aboveground insects, which have far more options for active dispersal. Still, this type of information may be crucial for designing effective biological control strategies, for example, when determining to rely on specialists or generalists for nematode control (Lehman and Reid, 1993; Snyder and Ives, 2001; Symondson *et al.*, 2002). Other examples may be selective suppression of plant-parasitic nematodes by entomopathogenic nematodes, *Heterorhabditis bacteriophora* and *H. indica*, with no adverse effect on free-living nematodes (Somasekhar *et al.*, 2002). Or evidence that bacterial-feeding nematodes make a greater contribution to the diet of predacious nematodes than do plant-feeding nematodes. Increased numbers of predacious nematodes enhance the cycling of plant nutrients rather than reducing root herbivory (Yeates and Wardle, 1996).

Multitrophic approaches have been advocated for plant-parasitic nematodes (Kerry and Bourne, 1996; Sikora, 1992), but they have already been applied to entomopathogenic nematodes, for example, interactions with trapping fungi and their influence on entomopathogenic nematodes, root-feeding insects, and, finally, survival of bush lupines in coastal systems (Jaffee *et al.*, 1996).

Bacterivorous and fungal feeding nematodes play a major role in soil food webs and these have been included in developing soil food web theory (De Ruiter *et al.*, 1995; Moore *et al.*, 2003; Neutel *et al.*, 2002). However, plant-parasitic nematodes thus far have played a minor role in these detritus-focused food web models. While studies on suppressive soils would provide excellent examples on improving the application and development of ecological theory for belowground, very few of these studies have done so. The awareness of linkages between above- and belowground trophic interactions (Masters *et al.*, 1993; Van der Putten *et al.*, 2001; Wardle *et al.*, 2004) is showing that nematodes may stabilize or destabilize aboveground multitrophic interactions (Bezemer *et al.*, 2005). This shows that further development of aboveground multitrophic interaction theory requires that

belowground interactions, including root–nematode interactions, should be considered as well.

B. COMPARING NATURAL SYSTEMS, TROPICAL/ORIGINAL AGRICULTURE, AND INTENSIVE AGRICULTURE

The current large seed crops, such as wheat and rice, are derived from domestication of early succession species that would have been quite ephemeral, having traded off growth against defense (Diamond, 1997; Herms and Mattson, 1992). Probably, the choice of the early farmers, some 10,000 years ago, is influencing current agriculture and crop protection enormously. Breeding for high yield and palatability may have resulted in selection pressures on plant enemies, leading to the current range of pest nematodes; whether or not this may be true should still be investigated. A comparative approach of plant defense strategies against above- and belowground natural enemies and higher trophic levels, including early, mid, and late succession plant species, may help us to understand plant defense strategies in natural systems. Comparison of defense in wild plants with intensive, extensive, or biological farming systems in tropical and temperate regions might result in new approaches for crop protection strategies. For example, arbuscular mycorrhizal fungi may be more effective in soils with low phosphorus availability, but application in fertile soils could have neutral or even adverse effects. Similar assessments could be made for bacterial, fungal, or faunal natural enemies of plant-parasitic nematodes and variations therein.

C. BIODIVERSITY AND CROP PROTECTION

Soil faunal diversity is supposed to be greatest in temperate areas, owing to the quite rich C content in soils increasing food resources and also inducing habitat heterogeneity (Wardle, 2002). However, biodiversity of plant-parasitic nematodes in tropical agrosystems is greater than in temperate agro-systems (Fargette and Quénéhervé, 1988; Luc *et al.*, 1990; Mateille *et al.*, 1995; Quénéhervé and Fargette, 1992). The plant-parasitic nematode diversity in temperate natural areas is supposed to be greater than in temperate agro-ecosystems (Hanel, 2003). Therefore, the relatively low plant-parasitic nematode diversity in temperate agro-ecosystems seems to be due to intensive cropping practices, rather than to climate. Biodiversity may influence the functioning of soil systems, but effects that have thus far been measured show negative, neutral, and positive effects (Wardle, 2002; Wardle *et al.*, 2004). Great nematode diversity could enhance competitive interactions, thereby preventing outbreak events of individual plant-parasitic

nematode species (Brinkman *et al.*, 2005). Considering the soil diversity as a potential resource might help manage the overall pathogenicity of the total plant-parasitic nematode community (Cadet and Floret, 1999; Cadet *et al.*, 2002, 2003a; Rimé *et al.*, 2003).

Patterns in biodiversity of the natural enemies of plant-parasitic nematodes have been less well studied. Soils harbor a variety of microbial and faunal species that all may be involved in nematode control. The question is how these control factors interact and what may be the result for nematode population dynamics. Holling (1973) proposed the "soil resilience concept," defined as the soil capacity to recover functional and structural integrity after disturbance. In line with more recent biodiversity studies, other aspects, such as niche complementarity, which has been demonstrated for plant mixtures (Van Ruijven and Berendse, 2005) may also be of importance for the control of plant-parasitic nematodes by the community of soil organisms. However, whether or not diversity in these multispecies communities of natural enemies could be considered as redundancy, insurance, or resulting in idiosyncratic nematode control when reducing diversity requires further studies.

V. DISCUSSIONS

Soils that are suppressive to plant-parasitic nematodes and other soil-borne diseases may be called "healthy" from a crop protection point of view, although soil suppressiveness may refer to a variety of different mechanisms, ranging from prevention of pathogen establishment, the presence of pathogens that do not become harmful, or to initial increase and subsequent decrease of pathogen incidence (Baker and Cook, 1974). Soil suppressiveness may or may not relate to soil biodiversity.Suppressions may be general, or specific, for example, due to the presence of biological control organisms (Cook and Baker, 1983). Plant pathologists describe suppressive soils as soils where plant disease is not expressed (Alabouvette, 1986) or considerably decreased as in the well-known Take-All Decline (Cook and Weller, 1987) despite the presence of virulent pathogens. For plant nematologists, a suppression effect results in the decrease of nematode populations by natural enemies exclusively (Kerry and Jaffee, 1997). In the first case, suppression involves complex mechanisms including abiotic as well as biotic factors. In the second one, suppression corresponds to specific biotic interactions.

Resilience (of which suppression is one component), stability, large biodiversity, and active nutrient cycles are all attributes of "soil health" (Elliott and Lynch, 1994). If we assume that too intensively managed agricultural

soils are endangered soils (sick soils), such a concept represents an approach and a new challenge toward soil quality restoration in modern agriculture (Swift, 1994). In highly disturbed environments, such as high input agricultural systems, monitoring soil diversity toward some recovery of more complex top-down, bottom-up, and horizontal (inter- and intraspecific competitive) interactions may lead to more sustainable ways of management. In that respect, it is essential to know the mechanisms of the interactions within or involving nematode communities and their implications for population regulation, since such interactions will contribute to save or partly restore resilience and sustainability.

A widespread dogma is that tropical crops suffer more from nematode damage than those of temperate regions (Luc *et al.*, 1990). This also requires evaluation for the lessons it may have for sustainable control. In developing countries of the tropics and subtropics, crop yields are mostly low due to the erosion of soils and low natural fertility of soils. In such conditions, nematode infestations may not be the principal cause of poor crop growth but the damage they cause can be considerable. Moreover, in traditional agro-ecosystems, the requirement for food production to be resilient to multiple stresses has favored the development of a broad range of plant species of high genetic diversity in complex agro-ecosystems involving mixed cropping, rotations, shifting cultivation. Such complexity may have led to the development of complex plant-parasitic nematode communities. Control methods targeting specific nematodes are then not very effective in reducing nematode damage and only nonspecific approaches (chemical nematicides) can be predicted to be generally effective. Such complex agro-ecosystems may be more similar to natural ecosystems than the systems of intensive agriculture.

The lesson from current control practices appears to be that cultivated plants suffer much nematode damage. Nevertheless, many crops, known to be susceptible to one or more nematode species, are grown often without nematodes being recognized as limiting factors. Such crops include fruit, vegetables, cereals, and others, and it seems that when damage does occur it is a consequence of some particular features of an agricultural system rather than a general feature of agriculture.

Likewise, from a consideration of top-down nematode control mechanisms we conclude that large-scale nemato-stasis—the control of particular species by specific or general antagonists—is unlikely to occur. Interactions are more likely to be important at smaller scales. The important lesson is that we have to define these dynamics at smaller scales than has so far been attempted and a major constraint for quantifying nematode population dynamics is the lack of precision of nematode population density estimates and those for their natural enemies (Kimpinski and Sturz, 2003).

VI. CONCLUSIONS

Plant-parasitic nematodes are serious pests in agriculture causing much economic damage, while driving vegetation processes (succession, diversity) in natural plant communities.

Reported nematode effects in natural plant communities are highly variable between studies.

It is not sure whether and, if so, why nematodes are less aggressive in nature: this may be due to invisible effects (e.g., by competition or control by predators), or due to more genetic variability (Red Queen processes), or less aggressive nematodes (resistance breeding effect), and more diversity (diversity-functioning effect).

In natural systems the diversity between and within top-down and horizontal (competition) nematode control effects may lead to insurance or resilience. Nematology research would benefit from a more conceptual multitrophic interactions approach.

Comparative assessment may reveal the importance of effects of top-down, horizontal, and bottom-up control of plant-parasitic nematodes in nature; the advantage of natural systems is that plants, plant-parasitic nematodes, and their natural enemies may have coevolved considerably longer than multitrophic interactions with crop plants.

Natural systems may be compared with agro-ecosystems with various degrees of intensity of disturbance to analyze the consequences of cultivation for plant-parasitic nematode control; this may result in improved integrated nematode control, which contributes to enhancing sustainability of agriculture.

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ALGORITHMS DETERMINING AMMONIA EMISSION FROM BUILDINGS HOUSING CATTLE AND PIGS AND FROM MANURE STORES

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Livestock excreta and manure stored in housing, in manure stores, in beef feedlots, or cattle hardstandings are the most important sources of ammonia (NH_3) in the atmosphere. There is a need to quantify the emission, to assess the effect of emission on NH_3 and ammonium (NH_4^+) deposition to ecosystems and on the health risks posed by NH_4^+ -based particles in the air. To obtain a reliable estimate of the emission from these sources, the processes involved in the transfer of NH_3 from the manure to the free atmosphere have to be described precisely. A detailed knowledge of the processes of NH_3 transfer from the manure and transport to the free atmosphere will contribute to development of techniques and housing designs that will contribute to the reduction of NH_3 emission to the atmosphere. For this reason, this review presents the processes and algorithms involved in NH_3 emission from livestock manure in livestock buildings and manure stores for pigs and cattle. Emission from poultry buildings and following land application of manure, although significant sources of NH_3 , have been reported in earlier reviews and are not included here.

A clear description of the features that contribute to the total NH_3 emission from buildings will include information on stock class, diet and excreta composition, the distribution of emitting surfaces and knowledge of their mass transfer characteristics in relation to the building as a whole, as well as environmental variables. Other relevant information includes the quantity and composition of excreta produced by different classes of livestock and the influence of feeding regime; the influence of environmental variables on the production of NH_3 from excreta; how excreta is distributed and managed in livestock buildings;

and factors that affect mass transfer of NH₃ in the building to the atmosphere outside. A major factor is the pH of the manure. There is a great need for algorithms that can predict pH as affected by feeding and management. This chapter brings together published estimates of NH₃ emissions and abatement techniques, and relates these to the factors listed above (excreta, NH₃ production, building, and mass transfer).

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ABBREVIATIONS

<i>A</i>	area of the NH ₃ emitting source
<i>D</i>	mass diffusion coefficient, m ² s ⁻¹
<i>F</i>	NH ₃ flux, kg m ⁻² s ⁻¹
HAc	protonated acetic acid (CH ₃ COOH)
<i>K_t</i>	mass transfer coefficient, m s ⁻¹
<i>K_H</i>	Henry's constant
<i>K_N</i>	equilibrium constant between NH ₄ ⁺ and NH _{3,L}
NH ₃	concentration of NH ₃ , g m ⁻³
NH _{3,A}	ambient concentration of gaseous NH ₃
NH _{3,G}	concentration of gaseous NH _{3,G} in equilibrium with NH _{3,L} in solution
NH _{3,L}	ammonia (NH ₃) in solution in equilibrium with NH ₄ ⁺
NH ₄ ⁺	ammonium (NH ₄ ⁺) in solution in equilibrium with NH _{3,L}
NO ₃	nitrate
N ₂ O	nitrous oxide
NO	nitrogen oxide
N ₂	free nitrogen gas
<i>M</i>	molecular weight, g mol ⁻¹
<i>P</i>	atmospheric pressure, atm
pH	manure surface pH
<i>r</i>	mass transfer resistance, s m ⁻¹
<i>r_a</i>	resistance in turbulent layer above the surface of manure in outside stores or surface of unconfined sources [s m ⁻¹]
<i>r_b</i>	resistance in the laminar boundary above the surface of manure in outside stores or surface of unconfined sources [s m ⁻¹]
<i>r_c</i>	resistance above the surface layer of manure in outside stores or surface of unconfined sources [s m ⁻¹]
<i>r_n</i>	mass transfer resistance at <i>n</i> th layer of transfer process, s m ⁻¹

Re	Reynold's number
Sc	Schmidt number
Sh	Sherwood number
T	temperature of slurry, °C
TAN	total ammoniacal nitrogen = $[NH_4^+] + [NH_{3,L}]$
TIC	total inorganic carbon = $[CO_2] + [HCO_3^-] + [CO_3^{2-}]$
u	airflow affected by ventilation or wind
V	ventilation rate, $m^3 s^{-1}$
VFA	volatile fatty acids C_1 – C_5
$\sum_j v_{ij}$	diffusion volumes for molecules of species j

Subscripts

a	air in the open space of the animal house
i	number of emission sources
o	opening
of	slatted floor
r	room
s	surface of contaminant source
sf	solid floor
sl	slurry channel
t	all contaminant surfaces/sources
v	ventilation
w	wall of slurry channel
1 ... n	layer of transfer process

I. INTRODUCTION

Agriculture is recognized as the major source of atmospheric ammonia (NH_3), contributing 55–56% of the global NH_3 emissions (Bouwman *et al.*, 1997; Schlesinger and Hartley, 1992). Inventories have shown that animal housing, stored animal manure, and exercise areas account for about 69–80% of the total emission of NH_3 in Europe (ECETOC, 1994; Hutchings *et al.*, 2001).

Close to the source, NH_3 gas is deposited rapidly on vegetation or soil (Asman and van Jaarsveld, 1991). However, NH_3 readily combines with sulfate (SO_4^{2-}) and may combine with nitrate (NO_3^-) to form particulates containing ammonium (NH_4^+) (Asman *et al.*, 1998). Particulate NH_4^+ , and to a lesser extent NH_3 , may be transported over long distances. Deposition of NH_3 or particulate NH_4^+ to land or water may cause acidification and

eutrophication of natural ecosystems (Fangmeier *et al.*, 1994; Schulze *et al.*, 1989). Furthermore, NH₃ emissions play a role in the formation of PM_{2.5} and PM₁₀, airborne particulates that can be a health hazard (Erisman and Schaap, 2004; McCubbin *et al.*, 2002). Consequently, ceilings on the annual NH₃ emissions were included in the Gothenburg Protocol United Nations convention on long-range transboundary air pollution (CLRTP, United Nations, 2004), and in the EU National Emissions Ceilings Directive (NECD) (EEA, 1999).

For farmers, the loss of NH₄⁺ via volatilization from animal houses, hardstandings, and manure stores will reduce the fertilizer value of animal manure applied in the field (Sørensen and Amato, 2002). In addition, the variability of NH₃ emission will cause variability and uncertainty in the fertilizer efficiency of the manure, reducing farmers' confidence in manures as a source of nitrogen (N) for crops. This may lead them to over supply the crops with N, risking a reduction in crop quality and increasing losses of N to the environment by leaching of nitrate and emission of nitrous oxide (N₂O) and dinitrogen (N₂) and a potential risk of a reduction in crop quality.

Estimates of national emissions should be reliable and generated by a commonly accepted methodology for the inventory of NH₃ emission. Consequently, the CLRTP and NECD require inventories to be constructed in accordance with the Emissions Inventory Guidebook (EIG). For NH₃ this specifies simple (Tier 1) and detailed (Tier 2) methodologies. Both these methodologies are based on annual emission factors, for example, yearly emission per animal or per kg N deposited in animal housing. However, two considerations suggest that a more dynamic, process-based (Tier 3) approach will be increasingly necessary. Firstly, the atmospheric dispersion models used to assess the geographic distribution of NH₃ deposition require emissions estimates at a much higher temporal resolution (Gyldenkerne *et al.*, 2005; Pinder *et al.*, 2004). Secondly, abatement techniques applied through changes in animal feeding or in animal housing will often modify the physical and chemical nature of the manure that then passes through storage and is applied to the land.

Consequently, algorithms or models for estimating emission of NH₃ from animal manure and mineral fertilizers applied in the field have been the subject of a number of recent articles (Genermont and Cellier 1997; Harrison and Webb, 2001; Huijsmans and De Mol, 1999; Misselbrook *et al.*, 2004; Sommer *et al.*, 2003). Emission of NH₃ from poultry manure has also been studied and reviewed thoroughly (Carlile, 1984; Groot Koerkamp, 1994; Groot Koerkamp and Elzing, 1996; Groot Koerkamp *et al.*, 1995, 1998a, 1999a,b; Kroodsma *et al.*, 1988). Consequently, the present review focuses on the emission of NH₃ from buildings housing livestock, cattle feedlots, other impermeable yard areas (hardstandings, exercise areas), and stored animal manure. This review will focus on housing and storage systems in

Europe and North America, because very few studies of NH_3 emission from housing and manure stores have been conducted in Asia, Africa, South America, or Oceania.

Our intention is to review the literature for the purpose of describing the processes of most importance for the emission of NH_3 . The focus is on developing algorithms that may be used in models for the calculation of the emission from cattle and pig housing, cattle feedlots, hardstandings, and animal manure stores. Thus, the algorithms describing the transport and chemistry processes should be able to account for European and North American farming systems, and should also show the differences in farming systems between regions in North America and Europe. Furthermore, the calculation should encompass different livestock categories and account for seasonal climatic variations, because the results of such calculations are used to assess the effect of emission on deposition to ecosystems (Gyldenkærne *et al.*, 2005) and on the health risks of NH_4^+ -based particles in the air.

II. LIVESTOCK FARMING PRACTICES

The design of animal housing, and methods of manure storage and manure handling reflect the large differences in climate and production objectives across Europe and North America. Housing has been developed to give shelter and provide a comfortable and dry environment for animals, with the purpose of increasing production and to facilitate feeding. In some dry climates, such as the North American prairies, there is less need of shelter, and both dairy cows and calves for beef production are raised in open feedlots even at temperatures less than -20°C . In Europe, the most important types of housing systems are loose housing versus tied housing systems and liquid manure versus solid manure systems. For cattle, loose housing systems are typical except for some Alpine and Scandinavian countries where traditional tied housing systems are still quite common. For pigs, loose housing is standard with the exception of housing for sows. Nevertheless, systems where the sows are confined will be abolished in the near future for animal welfare reasons. The proportion of the total manure produced in the form of liquid manure/slurry and solid manure varies considerably between countries (Burton and Turner, 2003; Menzi, 2002). The proportion as liquid manure/slurry is greatest in the Netherlands (around 95%) and least (below 20%) in some Eastern European countries. In general, the proportion of liquid manure/slurry is large ($>65\%$) in most Western/Central European countries and smaller in Eastern Europe as well as the United Kingdom and France. For animal welfare reasons there is a trend toward more solid manure systems in many countries.

Animal manure collected in housing systems has to be stored for a period inside or outside the housing until it can be transported to its final destination, usually field spreading. The size and nature of this storage depends to a large extent on the value of the nutrients in the manure and on the regulatory climate. Often, the storage capacity is designed to allow timely spreading of the manure in the field, that is, during the growing season when the crop can utilize the plant nutrients.

A. HOUSING

Animal manure from housing is a mixture of feces and urine, bedding material (straw, wood shavings, sawdust, sphagnum, etc.), spilt feed and drinking water, and water used for washing floors. Housing systems are often adapted to the category of housed animal such as calves, dairy cows, sows, fatteners, and so on. Table I presents the terminology for the most typical animal categories.

Most cattle buildings are naturally ventilated. In the United States fans for open airflow are common but closed tunnel ventilation systems are also

Table I
Definitions of Cattle and Pig Categories (RAMIRAN Glossary of Terms on Livestock Manure Management, Arogo *et al.*, 2003; Pain and Menzi, 2003)

Cattle or pigs	Category	Definition	Weight interval (kg)	Age
Cattle	Fattening calves		Birth to ca. 200	Usually <0.5 year
	Breeding calves		60 to ca. 300	<1 year
	Heifers	Female breeding cattle to first calving	Up to 450–550	1–3 years
	Dairy cows	Milked cows	Average 500–750	After 1st calving
	Beef cattle	Cattle held for beef production	Up to 450–550	Up to 14–30 months, depending on system
Pigs	Sows	Sows and piglets to weaning	Piglets <7–9	Sow from first litter
	Weaners	Weaned piglets until start of fattening	From 7–9 to 25–30	From 3–5 weeks to 10–12 weeks
	Fattening pigs		From 25–30 to 90–110	10 to ca. 25 weeks
	Growers	Fattening pigs <60 kg	25–60	
	Finishers	Fattening pigs >60 kg	60 to 90–110	

emerging. In cattle houses based on slurry, excreta are collected from below the slatted floor or in tied housing systems in a gutter behind the animals. The slatted floor area may cover the entire floor or be restricted to the walking alleys or the area behind tied cows (Table II). Some buildings with slurry systems are also equipped with automated scrapers. In the buildings with solid floor resting areas and slatted walk ways, the solid resting area may be strewn with straw, sawdust, wood shavings, peat, etc. (Menzi *et al.*, 1998; Monteny and Erisman, 1998). Calves for beef production are often housed in animal buildings with a solid floor covered with bedding, in which urine and excreta are deposited. Such systems also have an increasing importance in Europe for larger cattle (heifers, beef cattle, suckling cows) for animal welfare reasons. In a large part of buildings with tied dairy cows, the excreta are separated into solid manure (farmyard manure; FYM), mainly containing feces and straw, and liquid manure, which is a mixture of water, urine, and dissolvable fecal components. The area of the soiled and thus emitting surface per cow is typically 3–5 m² for loose housing systems and 1–1.5 m² for tied housing systems.

Pig houses often have forced or mechanical ventilation systems. The floor type determines the management of manure. Pig manure can be handled as a liquid or solid. Buildings with slatted floors are common, with manure falling into channels or stores below the floor. The manure management in these buildings is mainly via deep pit, pull plug, pit recharge, and flushing systems (Arogo *et al.*, 2003). The frequency of manure removal varies from several times a day, up to monthly intervals. With respect to NH₃ emissions the manure removal system (e.g., type of channel, removal frequency) is more important than the housing system. Some pig housing systems have been developed with partially or fully solid concrete floors strewn with straw or sawdust to improve the welfare of the pigs. Typically, the solid manure is removed manually or with front loaders at monthly intervals.

B. MANURE STORES

The EU Nitrate Directive (EC, 1991) sets limits for the period of time during which manure application is prohibited. Consequently, animal manure storage capacity should be sufficient to store manure for at least that period and most of European countries have guidelines concerning the minimum period of storage for manure, especially for liquid manure/slurry. The guidelines aim to ensure sufficient storage capacity to allow manure to be spread on land only at times when there is a demand for nutrients by crops and little risk of environmental impacts (e.g., losses to water or air, soil compaction, etc.). The actual average storage capacity for liquid manure or slurry is around 6 months in many countries but longer in Scandinavian

Table II
Housing Systems for Cattle and Pigs and the Related Manure Store (From Arogo *et al.*, 2003; Hutchings *et al.*, 2001; Pain and Menzi, 2003)

Animal	Type of housing	Flooring/manure type	Storage time	Animal category
Cattle	Cubicle, solid floor	Solid floor; slurry or slurry and solid manure	Regular removal	Dairy cattle
Cattle	Cubicle, partly slatted floor	Resting area solid floor; walk-aleys with slatted floor; slurry or slurry and solid manure	Solid floor regular removal, slatted floor continuous or regular removal but stores always contains some slurry	Dairy cattle
Cattle	Fully slatted	All floor slatted	Storage below slat or continuous or regular removal but stores always contains some slurry	Beef cattle
Cattle	Tied stalls, slurry system	Tied concrete standing area with channel covered by a grid at rear of animals to collect excreta	Continuous or regular removal, but stores always contains some slurry	Dairy cows, heifers
Cattle	Tied stalls, liquid/solid manure system	Tied concrete standing area; daily removal of solid manure; liquid drained by gutter or stored in channel behind animals with channel covered by a grid at rear of animals to collect excreta	Channel with continuous or regular removal, but stores always contain some liquid manure	Dairy cattle
Cattle	Deep litter	Solid floor with deep litter; solid manure	Accumulated for several months, stored before land application or spread directly	Beef cattle
Cattle	Deep litter, sloped floor	Deep litter on sloped floor; solid manure	Accumulated; regular removal of some solid manure at the bottom of the slope	Beef cattle
Pigs	Slurry systems	Fully or partly slatted floor; flush discharge	1–24 h	Sows, fatteners, piglets
Pigs	Slurry systems	Fully or partly slatted; pit discharge	4–7 days	Sows, fatteners, piglets
Pigs	Slurry systems	Fully or partly slatted; pull plug discharge	7–14 days	Sows, fatteners, piglets
Pigs	Slurry systems	Fully or partly slatted; deep pit below animals	3–6 months	Sows, fatteners
Pigs	Deep litter system	Solid floor with deep litter; solid manure	3 months	Sows, fatteners, piglets

countries and shorter in some Southern and Eastern European countries. For solid manure the storage capacity varies from 2 to 12 months. For most countries it is less or equal to that for liquid manure/slurry (Burton and Turner, 2003; Menzi, 2002; Smith *et al.*, 2000, 2001a,b).

Liquid manure/slurry is mostly stored in tanks made from concrete or enameled steel sheets outside the livestock houses, except for the Netherlands, Ireland, and Norway where slurry stores may be partly below the slatted floor of the animal building and partly outside in slurry tanks (Burton and Turner, 2003; Menzi, 2002). Lagoons and lined ponds are the major storage system in North America and are also in the United Kingdom (Smith *et al.*, 2000, 2001b) and some Southern and Eastern European countries. Currently, efforts are being made to replace lagoons with tanks in many European countries. Slurry lagoons and tanks are normally not covered, unless there has been a tradition of covering liquid manure stores (e.g., in Switzerland) or covers are required by law to reduce emission of NH_3 and odor (e.g. in Denmark, Finland, and the Netherlands), or to exclude rainfall. The liquid manure/slurry is usually homogenized (stirred) in the tank prior to application.

Solid manure is usually stored in uncovered heaps on concrete pads, which in most countries and cases are designed so that drainage is collected. Storage of solid manure in the field is reported only from Denmark, Italy, some Eastern European countries and the United Kingdom.

C. FEEDLOTS AND EXERCISE AREA

Most feedlots are situated in areas with a semiarid climate and common system for beef cattle in the United States and some Mediterranean countries (e.g., Spain). Feedlots differ from housing, not only due to the absence of a roof but also because the manure is emptied from the feedlots only at 2- to 3-year intervals. The manure will typically be transported directly to the field and soon after spread on the soil, thus, the manure is *de facto* stored in the feedlot.

Hardstandings are defined as unroofed paved or concrete areas. Examples include areas (i) outside the milking parlor, where the dairy cows congregate prior to milking, (ii) exercise yard for dairy cattle kept in tied stalls as is required in some countries (e.g., Switzerland) for animal welfare reasons, and (iii) other feeding or handling areas. The amount of urine and feces deposited on the hardstanding depends on the length of time the animals are present (and to some extent their activities). Hardstandings are typically cleaned by scraping (handheld or tractor-mounted), although the frequency and effectiveness of cleaning will vary from farm to farm. Less commonly, yards may be washed. The efficiency of removal is greater by

washing than by scraping, when some residue remains and becomes a source of NH₃ emission. The extent and frequency of use of such areas in England and Wales is given by Webb *et al.* (2001) together with the mean area per animal (e.g., 1.7 and 3.4 m² per animal for dairy cow collecting and feeding yards, respectively). However, there is a large range in the areas and usage of hardstandings.

III. SYSTEM ANALYSIS

A. NITROGEN FLOW

Nitrogen flow in the animal production system (Fig. 1) is part of the N cycle, which is one of the most important nutrient cycles found in terrestrial ecosystems. Nitrogen is used by living organisms to produce complex organic molecules such as amino acids, proteins, and nucleic acids. Cattle and pigs obtain their N compounds from feed and grazing, and convert them into animal meat and milk; the surplus is then excreted in the form of urea and organic N by the animal. Organic bedding materials, such as straw or sawdust, employed in the animal production process, may add organic N and carbon (C) to the manure. Although the major part of N in manure is applied in the field as a fertilizer for crops, a part of it is lost to the atmosphere due to NH₃ emissions as oxidized or reduced N from manure and manure-applied soil.

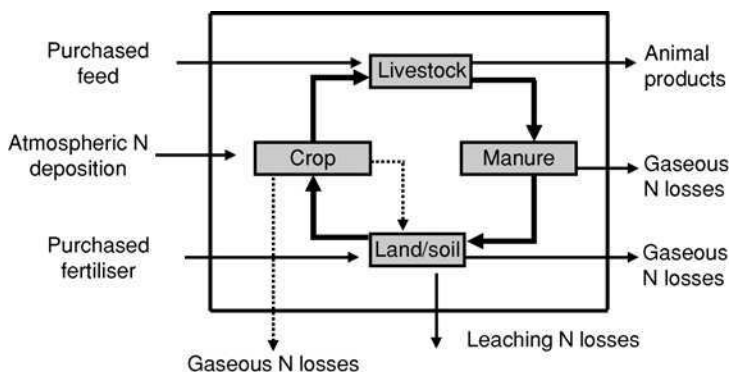


Figure 1 Nitrogen flow in a livestock farming system.

B. AMMONIA AND MANURE

The sources of NH_3 emission from the livestock production system are N excreted in the form of urea and organic N by livestock in the housing or outdoor areas (Fig. 2). Animal housing, outdoor holding areas, and manure storage are an integrated system, with N cascading from one source to another. Ammonia lost from an upstream source (e.g., housing) is not subsequently available for loss from manure storage.

Organic N may be transformed to $\text{NH}_4\text{-N}$ ($\text{TAN} = [\text{NH}_3] + [\text{NH}_4^+]$) by microorganisms (mineralization) or vice versa (immobilization), depending on whether the manure has a low or high C:N ratio. This addition or removal of TAN will tend to increase or decrease NH_3 concentrations accordingly. Bedding material usually has a high C:N ratio relative to animal excreta, thereby promoting immobilization (Kirchmann and Witter, 1989).

TAN may also be converted to NO_3 and N may be lost as N_2O , NO, or N_2 during nitrification or denitrification (Oenema *et al.*, 2001).

C. CONCEPTS OF AMMONIA RELEASE, EMISSION, AND DISPERSION

Diffusion and convective mass transport is involved in the transport of NH_3 from animal manure to the free atmosphere. The transport can be divided into two closely related processes: (i) NH_3 transfer over the interface of the manure–air boundary layer and (ii) transport from this interface to the free atmosphere (Figs. 2 and 3). The transfer over the manure-to-atmosphere interface may be referred to as “release.” An NH_3 concentration gradient is essential for the release and transport. In most cases NH_3 release from the manure to the atmosphere equals the NH_3 emitted from most sources described in this article, but for example in animal housing, NH_3 may be absorbed in filters and the amount released from the manure may therefore be larger than the amount emitted from the animal house. Ammonia dispersion is the process used to transport the emitted NH_3 either short or long distances through the open atmosphere to the NH_3 sink. Ammonia dispersion has been studied by various authors (Asman and Janssen, 1987) and is not within the scope of this review.

Emission of NH_3 from manure follows the transport of NH_3 from the surface of an ammoniacal solution of dissolved NH_3 ($\text{NH}_{3,\text{L}}$) and NH_4^+ to the atmosphere. The solution containing TAN can be in the surface of stored slurry, urine patches on the floor, and slats in animal houses or outdoor animal-holding areas such as hardstandings and feedlots. The source may also be the liquid phase in solid manure containing TAN, which are solid manure stored in heaps, deep litter covering concrete floors, or litter on soil surfaces in beef cattle feedlots.

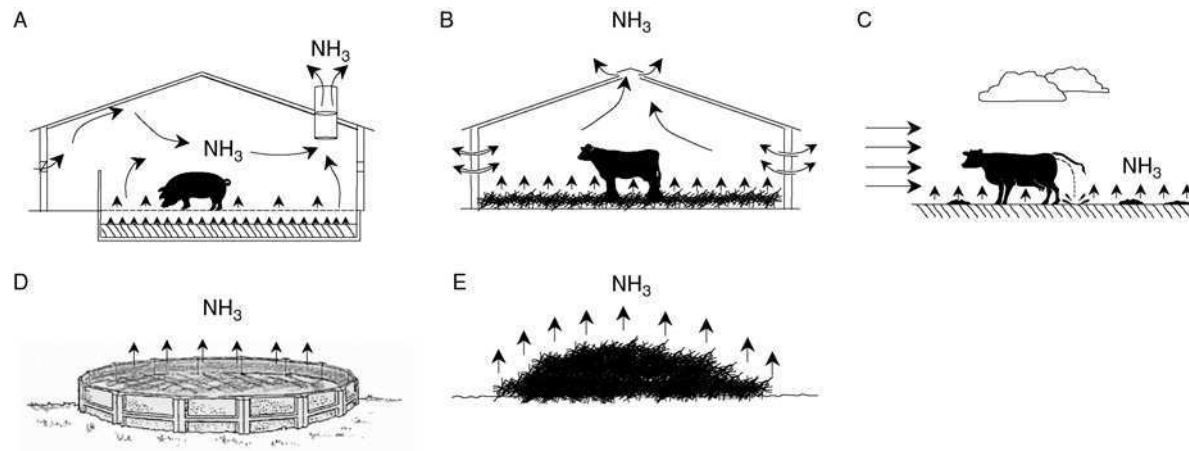


Figure 2 Nitrogen emission from (A) mechanically ventilated livestock building, (B) naturally ventilated building, (C) feedlot, (D) liquid manure storage, and (E) solid manure.

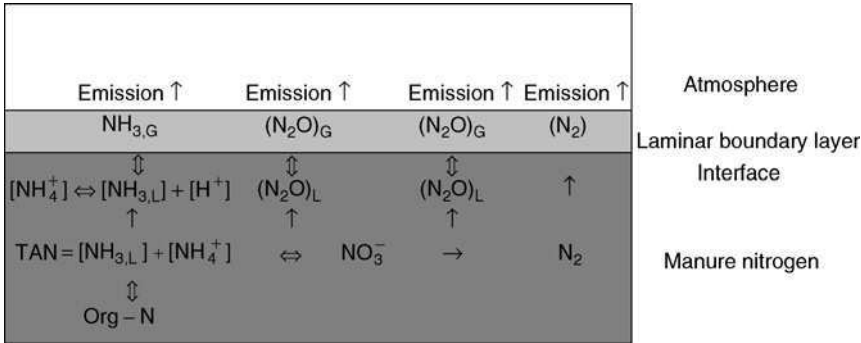


Figure 3 Nitrogen transformation in liquid manure and mass transfer of NH_3 from liquid manure to the free atmosphere.

The instantaneous NH_3 release is the function of the concentration of NH_3 ($\text{NH}_{3,\text{G}}$) in the air in immediate contact with $\text{NH}_{3,\text{L}}$ in the ammoniacal solution. The equilibrium of $\text{NH}_{3,\text{G}}$ with $\text{NH}_{3,\text{L}}$ is controlled by the Henry's constant (K_{H}). The $\text{NH}_{3,\text{L}}$ concentration is a function of the chemical composition of the solution and transformations within the manure that either increase or decrease the TAN concentration in the liquid. The rate of emission is further determined by the concentration gradient and resistance to NH_3 transport between the air in immediate contact with the emitting surface and the free atmosphere as controlled by atmospheric transport processes and barriers to the transport. The air above the surface can be envisaged as a laminar or turbulent-free layer close to the surface and, above this, a turbulent layer. Ammonia gas at the liquid–air interface is transported through the laminar layer by molecular diffusion and then in most cases through a turbulent layer to the free atmosphere by turbulent diffusion and advection. This review presents in principle three different compartments: (i) animal houses, beef cattle feed lots, and manure stores without covers, (ii) covered manure stores, and (iii) hardstandings (Fig. 2). In each case NH_3 release and transport has to be described differently.

In open-air feedlots and manure storage without cover, all the NH_3 released will be transported to the free atmosphere. Therefore, NH_3 release equals NH_3 emission. Ammonia release is largely affected by available TAN, equilibrium processes, and weather conditions. The release of NH_3 from the stored manure may be affected by the formation of a crust, or PVC cover floating on the liquid manure, or a roof over the liquid manure store due to an increase in the NH_3 concentration in the air above the manure, which reduces the concentration gradient and emission of NH_3 . Solid manure may be covered by sphagnum or a PVC cover. Both crusts and PVC covers may

be viewed as diffusion barriers to the NH₃ transport. In animal housing the building structure and air movement will affect the released NH₃ from the manure surface and transport of NH₃ to ventilators or openings and to the free atmosphere. As mentioned above, filters may be installed in animal houses and emission will consequently be lower than release of NH₃ from the manure.

IV. RELEASE AND TRANSPORT MODEL

The physics of NH₃ transport from all sources can be depicted using a resistance approach. The calculation of the resistance to transport will differ between environments and between different designs of animal housing, manure storage, and feedlot. The chemical reactions in the liquid and the release of NH₃ to the air immediately above the manure can also be described by one set of equations, although the processes affecting the chemistry may differ among sources of NH₃ emission.

Emission of NH₃ from all farm sources (i.e., housing, cattle feedlots, hardstandings, stored manure) may be calculated with the following equation:

$$F_{\text{NH}_3} = \frac{1}{r_1 + r_2 + \dots + r_n} A \times (\text{NH}_{3,\text{G}} - \text{NH}_{3,\text{A}}), \quad (1)$$

where A is the surface area of the NH₃ source, r_1 – r_n are the resistance to transport of NH₃ between the surface and the free atmosphere, and are affected by ventilation or wind and surface parameters (i.e., roughness), $\text{NH}_{3,\text{G}}$ is the atmospheric concentration of NH₃ in the air layer immediately above this surface, and $\text{NH}_{3,\text{A}}$ is the ambient atmospheric NH₃ concentration. However, it is usual to ignore $\text{NH}_{3,\text{A}}$ because the concentration is low compared with $\text{NH}_{3,\text{G}}$. Instead of the resistance approach one may include a transfer coefficient in the equation (Arogo *et al.*, 1999; Xue *et al.*, 1999):

$$F_{\text{NH}_3} = K_t \times A \times \text{NH}_{3,\text{G}}, \quad (2)$$

where K_t is a transfer coefficient, which is the reciprocal of the sum of resistances:

$$K_t = \frac{1}{r_1 + r_2 + \dots + r_n}. \quad (3)$$

The release of NH₃ from the surface to the laminar air phase immediately above the liquid surface is driven by the differences in the atmospheric

concentration of gaseous NH_3 ($\text{NH}_{3,\text{G}}$) in equilibrium with liquid NH_3 ($\text{NH}_{3,\text{L}}$) in the surface liquid layer:



$$[\text{NH}_{3,\text{L}}] = \frac{[\text{TAN}]}{1 + [\text{H}^+]/K_{\text{N}}} \quad (5)$$

$$\text{NH}_{3,\text{G}} = K_{\text{H}} \frac{[\text{TAN}]}{1 + [\text{H}^+]/K_{\text{N}}} \quad (6)$$

Included in the equations is the TAN in the manure surface layers or soiled areas, the equilibrium between $\text{NH}_{3,\text{G}}$ in equilibrium with $\text{NH}_{3,\text{L}}$ is affected by Henry's constant (K_{H}), the equilibrium constant (K_{N}) between $[\text{NH}_4^+]$ and $[\text{NH}_{3,\text{L}}]$ and livestock slurry surface proton concentration $[\text{H}^+]$, $\text{pH} = -\log(\text{H}^+)$.

The NH_3 in solution ($\text{NH}_{3,\text{L}}$) is the product of the dissociation of NH_4^+ , which produces 1 mol of H^+ for each mole of NH_3 [see Eq. (4)]. The concentration of $\text{NH}_{3,\text{L}}$ is therefore related to both the concentrations of $[\text{TAN}]$ and $[\text{H}^+]$ in the solution. Further the concentrations of $\text{NH}_{3,\text{G}}$ and $\text{NH}_{3,\text{L}}$ are affected by temperature as the equilibrium constants K_{N} and K_{H} are exponential functions of the temperature of the solution (see Table III). Therefore, increasing temperature will increase the release of NH_3 from the manure.

A. SOURCES

In livestock housing based on slurry, the sources of NH_3 are the soiled area of the solid floor, slats, side of the slurry store, and the surface of the slurry stored below the slatted floor (Figs. 2 and 4). The physics and chemistry of these sources of NH_3 may differ; therefore, we have to split the housing compartment into NH_3 emission elements typical for each emitting surface (subscript: $s = 1 - n$). Having characterized the important elements, these may then be combined as appropriate to calculate NH_3 emissions from different housing types. The calculations should take into account that the period for which a surface may be a source can vary from a few hours for urine patches to continuous of below slat stored slurry. From the slurry surface below the slatted floor the resistances may encompass:

Table III
The most Used Equilibrium Constants for the Processes of NH₃ Transfer from Manure to the Atmosphere in Immediate Contact with Manure

Constant	Equation	Units	
Henry's constant	$\log K_H = -1.69 + \frac{1477.7}{T}$	No units	Hales and Drewes (1979)
Henry's law constant K_H for ammonia	$\ln(K_H) = 160.559 - \frac{8621.06}{T} - 25.6767 \times \ln(T) + 0.035388 \times T$	Atm. mol liter ⁻¹	Beutier and Renon (1978)
Henry's law constant K_H for ammonia	$\log K_H = 1.384 \times 10^{-3} \times 1.053^{(273-T)}$	No units	Hashimoto and Ludington (1971)
Acid base equilibrium constant K'_N for ammoniacal N	$\log K_N = -0.09018 - \frac{2729.92}{T}$	No units	Hales and Drewes (1979)

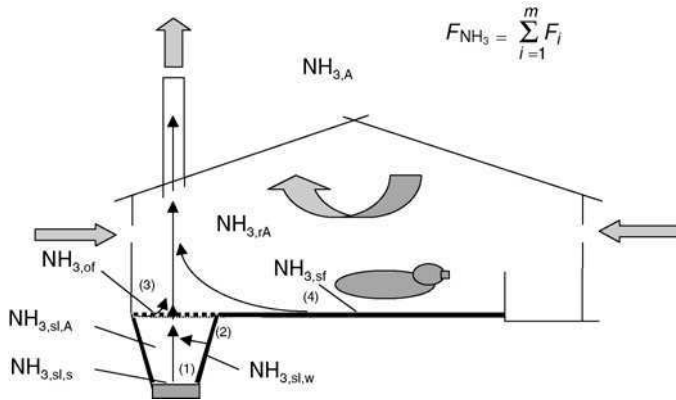


Figure 4 Conceptual model of NH_3 transport processes in animal houses. The emission of NH_3 from the house is given by the sum of emissions from each source in the animal house: (1) slurry surface, (2) soiled walls of the slurry channel, (3) slats above the slurry channel or store, and (4) soiled floor.

(i) transport from the surface to slats, (ii) through slats, (iii) from slats to opening of the house (including ventilation), and (iv) transport through the openings. Emission from the floor will include transport from the floor to the opening of the house (including ventilation) and through the openings. Ammonia emission from each source has to be summed to obtain the emission from the entire house. Emission models for housing with natural ventilation (e.g., most cattle housing) may be more complex, since the air exchange rate is dependent on both the thermal buoyancy forces and the wind pressures on the openings of the building. Although a temperature difference provides a buoyant force that induces ventilation in livestock buildings, the wind effects will contribute more to the air exchange as the wind speed increases. Furthermore, a ventilation opening may act as an inlet during one period and as an outlet during another period due to variations in the wind direction.

Ammonia emission from beef feedlots and hardstanding will have one source element, which is the area enclosed by fences, buildings, or walls (Fig. 5). The transport taken into account is from the surface to the open air. For liquid or slurry manure stores the approach will be similar, but the calculations may in addition have to account for transport through surface covers and crusts floating on the slurry or through a roof construction. For solid manure stores one may have to account for transport of air through the manure heap as well as surface process while estimating NH_3 emission.

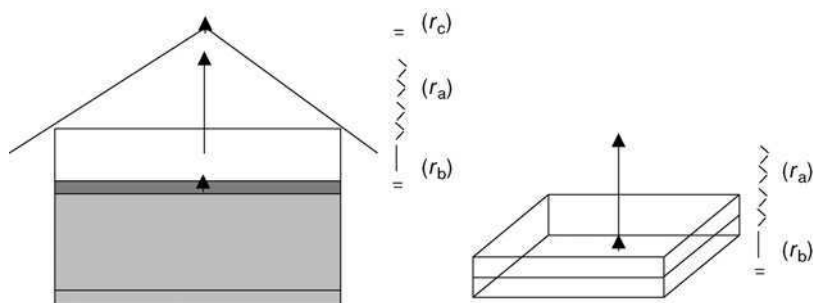


Figure 5 Transport processes of NH₃ emission from (left) stored liquid manure with a porous surface layer floating on the stored slurry and a roof and (right) a fenced feedlots (hardstanding).

B. TRANSPORT OF NH₃ IN ANIMAL HOUSES

Transport of released NH₃ is determined by indoor and outdoor NH₃ concentrations, building ventilation, and NH₃ abatement practices. The approach to transport presented here is based on the following assumptions: (i) the total emitted mass from the sources is transported into building airspace without any chemical or biological action during the transport; (ii) the mass diffusion and transfer at the boundary between two layers is in one direction; and (iii) the transport process may be divided into multilayer subprocesses. Generally, NH₃ mass flux per unit area from surface of a source to building airspace may be described as in Eq. (1).

Based on the assumptions stated above, the process is divided into n layers, that is, the atmospheric concentration of ammonia NH_{3,A} is equal to NH_{3,n} and the ammonia concentration immediately above a contaminant surface NH_{3,s} is equal to NH_{3,G} in Eq. (1), so we have

$$F_{\text{NH}_3} = K_1(\text{NH}_{3,G} - \text{NH}_{3,1}) = K_2(\text{NH}_{3,1} - \text{NH}_{3,2}) \\ = \dots = K_n(\text{NH}_{3,n-1} - \text{NH}_{3,n}). \quad (7)$$

Notice that (i.e., nomenclature used in the transport model is presented in the abbreviations list)

$$(\text{NH}_{3,G} - \text{NH}_{3,n}) = (\text{NH}_{3,G} - \text{NH}_{3,1}) + (\text{NH}_{3,1} - \text{NH}_{3,2}) \\ + \dots + (\text{NH}_{3,n-1} - \text{NH}_{3,n}). \quad (8)$$

Applying the analogy of the Ohm's law, combining Eqs. (7) and (8), we have

$$\frac{1}{K_t} = \frac{1}{K_1} + \frac{1}{K_2} + \cdots + \frac{1}{K_n} = r_1 + r_2 + \cdots + r_n \quad (9)$$

and the overall NH_3 mass transfer coefficient given in Eq. (3).

$$K_t = \frac{1}{r_1 + r_2 + \cdots + r_n}. \quad (10)$$

Transport from the sources in the animal building to the outdoor atmosphere may be simplified as presented in Fig. 4. The four major sources for NH_3 emission considered in the model for a livestock building are: (i) manure surface, (ii) sidewalls in slurry channels, (iii) surface of the slatted floor, and (iv) surface of the solid floor. The air volumes in the room space and in the headspace of a slurry channel are assumed to be fully mixed, except in the boundary layers near the source surfaces. The locations of animals in the building are not considered in the model.

An essential issue to consider when characterizing convection transfer is to determine whether the air motion in the boundary layer is laminar or turbulent. Surface friction and the convection transfer rates depend strongly on which of those conditions exists (Incropera and DeWitt, 1990). In a laminar boundary layer, air motion is highly ordered and it is possible to identify airflow dynamics. In contrast, air motion in a turbulent boundary layer is highly irregular and is characterized by velocity fluctuations. These fluctuations enhance the transfer momentum, energy, and surface friction as well as mass convection transfer rate.

In a slurry channel, the NH_3 emission flux from manure surface may be described as

$$\begin{aligned} F_{\text{sl},s} &= DSh_{\text{sl},s}(\text{NH}_{3,G} - \text{NH}_{3,\text{sl},a})/l_{\text{sl},s} = K_{\text{sl},s}(\text{NH}_{3,G} - \text{NH}_{3,\text{sl},a}) \\ &= \frac{1}{r_{\text{sl},s}}(\text{NH}_{3,G} - \text{NH}_{3,\text{sl},a}), \end{aligned} \quad (11)$$

where $r_{\text{sl},s} = l_{\text{sl},s} D^{-1} Sh_{\text{sl},s}^{-1}$ is the resistance in the boundary layer over the emission surface (m s^{-1}); D , is the NH_3 diffusion coefficient in air ($\text{m}^2 \text{s}^{-1}$); $\text{NH}_{3,\text{sl},s}$ and $\text{NH}_{3,\text{sl},a}$ are NH_3 concentrations at the slurry surface and in the headspace of the slurry channel, respectively. The diffusion coefficient of NH_3 in air may be calculated by an empirical relation developed by Fuller *et al.* (1966),

$$D = \frac{10^{-7}(273.15 + T)^{1.75}(1/M_{\text{NH}_3} + 1/M_{\text{a}})^{1/2}}{p[(\sum_{\text{NH}_3} v_i)^{1/3} + (\sum_{\text{a}} v_i)^{1/3}]^2} \quad (12)$$

where diffusion volumes for molecules of air, $\sum_{\text{a}} v_i$ and NH_3 $\sum_{\text{NH}_3} v_i$ have a value of 20.1 and 14.9, respectively (Fuller *et al.*, 1966). For laminar flow sh may be calculated as follows,

$$Sh = 0.644Re^{1/2}Sc^{1/3}. \quad (13)$$

For turbulent flow the following algorithm may be used.

$$Sh = 0.037Re^{4/5}Sc^{1/3}. \quad (14)$$

The transport of NH_3 from the surface of the slurry to the slats may be a combination of laminar flow in a boundary layer just above the slurry surface and turbulent flow between this boundary layer and the slats above the slurry channel.

The emission rate from the slatted floor of the slurry channels is related to the air exchange rate between the headspace in the channel and the room airspace. The air exchange is driven by the pressure variation at the openings of the slatted floor. The exchange rate depends on the ventilation rate, the airflow pattern in the room, turbulence level above the slatted floor, and opening area in the slatted floor. If we consider the mass transfer between the air in the headspace of the slurry channel and the air in the room space as independent of the convection transfer process from the slatted floor, and the air exchange rate in the slurry channel is V_{sl} , the NH_3 mass flux through the slat openings may be expressed as,

$$F_{\text{sl,o}} = \frac{V_{\text{sl}}}{A_{\text{sl,o}}}(\text{NH}_{3,\text{sl,a}} - \text{NH}_{3,\text{a,r}}) = \frac{1}{r_{\text{sl,o}}}(\text{NH}_{3,\text{sl,a}} - \text{NH}_{3,\text{a,r}}) \quad (15)$$

where $A_{\text{sl,o}}$ is opening area of slatted floor (m^2); $r_{\text{sl,o}} = A_{\text{sl,o}}/V_{\text{sl}}$, is the resistance of the slatted floor to emission from the slurry channels, s m^{-1} ; $\text{NH}_{3,\text{sl,a}}$ and $\text{NH}_{3,\text{a,r}}$ are NH_3 concentrations in air in the headspace in the slurry channels and in the room space, respectively. Here, the NH_3 concentration in the boundary layer at the surface of slats is assumed to be the same everywhere, the airflow rate into the headspace is equal to the airflow out.

The sidewalls in the slurry channel are also contaminant sources for NH_3 emissions. We may describe the emission in the same forms as in Eq. (11):

$$\begin{aligned} F_{\text{sl,w,s}} &= DSh_{\text{sl,w,s}}(\text{NH}_{3,\text{sl,w,s}} - \text{NH}_{3,\text{sl,a}})/l_{\text{sl,w,s}} \\ &= K_{\text{sl,w,s}}(\text{NH}_{3,\text{sl,w,s}} - \text{NH}_{3,\text{sl,a}}) \\ &= \frac{1}{r_{\text{sl,w,s}}}(\text{NH}_{3,\text{sl,w,s}} - \text{NH}_{3,\text{sl,a}}). \end{aligned} \quad (16)$$

Similar to Eq. (11), the NH_3 emission fluxes from the surfaces of slatted and solid floor may be estimated by

$$\begin{aligned} F_{\text{of,s}} &= \frac{DSh_{\text{of}}(\text{NH}_{3,\text{of,s}} - \text{NH}_{3,\text{a,r}})}{l_{\text{of}}} = K_{\text{of,s}}(\text{NH}_{3,\text{of,s}} - \text{NH}_{3,\text{a,r}}) \\ &= \frac{1}{r_{\text{of,s}}}(\text{NH}_{3,\text{of,s}} - \text{NH}_{3,\text{a,r}}) \end{aligned} \quad (17)$$

and

$$\begin{aligned} F_{\text{sf,s}} &= \frac{DSh_{\text{sf}}(\text{NH}_{3,\text{sf,s}} - \text{NH}_{3,\text{a,r}})}{l_{\text{sf}}} = K_{\text{sf,s}}(\text{NH}_{3,\text{sf,s}} - \text{NH}_{3,\text{a,r}}) \\ &= \frac{1}{r_{\text{sf,s}}}(\text{NH}_{3,\text{sf,s}} - \text{NH}_{3,\text{a,r}}) \end{aligned} \quad (18)$$

respectively.

The emission resistances $r_{\text{of,s}} = l_{\text{of,s}} D^{-1} Sh_{\text{of,s}}^{-1}$ and $r_{\text{sf,s}} = l_{\text{sf,s}} D^{-1} Sh_{\text{sf,s}}^{-1}$ are dependent on the characteristics of the airflow in the surface boundary layers above the slatted and solid floor. The maximum thickness of the boundary layers may be estimated by the following equation for laminar flow,

$$\delta_c = 5lRe^{-1/2}Sc^{-1/3} \quad (19)$$

and the following equation for turbulent flow,

$$\delta_c = 0.37lRe^{-1/5}. \quad (20)$$

The NH_3 mass flux through the exhaust openings of building ventilation may be described as:

$$F_{rv} = \frac{V_{rv}}{A_{rv,o}} (NH_{3,a,r} - NH_{3,a}) = \frac{1}{r_{rv,o}} (NH_{3,a,r} - NH_{3,a}) \quad (21)$$

where, V_{rv} is room ventilation rates ($m^3 s^{-1}$); $A_{rv,o}$ is the outlet opening area (m^2); $r_{rv,o} = A_{rv,o} / V_{rv}$, is resistance of the ventilation outlet to NH₃ mass flux from the room airspace to the atmosphere, $s m^{-1}$. The $r_{rv,o}$ value has effects on the emissions from all the NH₃ sources in the building envelope.

Summarizing the above analysis and with continuity of the mass flux transfer, we have the following equations to estimate NH₃ mass transfer coefficients from slurry channels, slatted and solid floors through the exhaust openings of the room to atmosphere:

$$K_{sl} = \frac{1}{r_{sl,s} + r_{sl,o} + r_{rv,o}} \quad (22)$$

$$K_{sl,w} = \frac{1}{r_{sl,w,s} + r_{sl,o} + r_{rv,o}} \quad (23)$$

$$K_{of} = \frac{1}{r_{of,s} + r_{rv,o}} \quad (24)$$

$$K_{sf} = \frac{1}{r_{sf,s} + r_{rv,o}}. \quad (25)$$

Therefore, the total NH₃ emission from a livestock building may be estimated by

$$F_{NH_3} = F_{sl}A_{sl} + F_{sl,w}A_{sl,w} + F_{of}A_{of} + F_{sf}A_{sf} \quad (26)$$

where

$$F_{sl} = K_{sl}(NH_{3,sl,s} - NH_{3,a}) = \frac{1}{r_{sl}} (NH_{3,sl,s} - NH_{3,a}), \quad (27)$$

$$F_{sl,w} = K_{sl,w}(NH_{3,sl,s} - NH_{3,a}) = \frac{1}{r_{sl,w}} (NH_{3,sl,s} - NH_{3,a}), \quad (28)$$

$$F_{of} = K_{of}(NH_{3,of,s} - NH_{3,a}) = \frac{1}{r_{of}} (NH_{3,of,s} - NH_{3,a}), \quad (29)$$

and

$$F_{sf} = K_{sf}(\text{NH}_{3,sf,s} - \text{NH}_{3,a}) = \frac{1}{r_{sf}}(\text{NH}_{3,sf,s} - \text{NH}_{3,a}). \quad (30)$$

In this approach, the most important issue is to determine the resistance parameters. The basic factors that affect the resistances are ventilation rate, outlet area, the airflow characteristics above the floors, air exchange rate in the slurry channel, and the airflow characteristics in the slurry channel. The ventilation rate may be estimated based on the CO₂ production model of the animals. The method may be applied to both mechanically and naturally ventilated buildings (Pedersen *et al.*, 1998; Zhang *et al.*, 2004). A major challenge for a naturally ventilated building is to accurately estimate the outlet area in windy conditions. For a mechanical ventilation system the ventilation rate may be achieved directly by measurement. Airflow characteristics above the floor and the factors that affect them can be found in the literature (Heber *et al.*, 1996; Strøm *et al.*, 2002; Zhang *et al.*, 1999). In many cases, the flow characteristics vary according to the ventilation systems, partition of pens, and density of the animals in the room. Temperature gradients between emission source and air space above the source may also affect the airflow due to the buoyancy effect (Zhang *et al.*, 2002). In an investigation of the mass transfer coefficient of ammonia in liquid pig manure and aqueous solution by Arogo *et al.* (1999), the turbulence caused by thermal buoyancy was reported. A high turbulence level may result in a reduced resistance to mass flow from the emitting surfaces by reducing the boundary layer thickness. To estimate the flow characteristics in the headspace of the slurry channel and the air exchange rate in the headspace, further research is needed.

C. TRANSPORT FROM UNCONFINED SOURCES

For NH₃ emission from unconfined slurry stores, beef feedlots, and hardstandings, a three-layer model (Hutchings *et al.*, 1996; Olesen and Sommer, 1993) can be applied. The layers are a surface layer affected by surface condition, a laminar airflow layer above the surface layer, and a layer where airflow is fully turbulent. K_t [see Eq. (3)] is defined as:

$$K_t = \frac{1}{r_a + r_b + r_c} \quad (31)$$

where r_a is the resistance in the turbulent layer above the slurry, r_b is the resistance in the laminary boundary layer (i.e., between the gas-liquid interface and the turbulent layer), and r_c is the resistance of the manure surface cover.

The resistance r_a in the turbulent layer is calculated as [according to van der Molen *et al.* (1990a); Padro *et al.* (1994)]:

$$r_a = \frac{\ln(l/z_0)}{Ku_*}. \quad (32)$$

The wind velocity profile above the slurry is described by the standard equation under neutral conditions (Monteith and Unsworth, 1990):

$$u_z = \frac{u_*}{k} \ln \frac{z}{z_0} \quad (33)$$

where u_z is the wind velocity at height z above the slurry surface, u_* is the friction velocity, z_0 is the roughness length, and k is von Karman's constant. The roughness length varies with surface characteristics and wind velocity. The typical roughness length of $z_0 = 1$ mm used for bare soils (van der Molen *et al.*, 1990b) is chosen because the physical structure of typical slurry surfaces resembles that of bare soils. ζ is a correction for the atmospheric stability, which depends on the Richardson number Ri (Padro *et al.*, 1994):

$$\zeta = \begin{cases} (1 - Ri)^{-2} & -0.1 \leq Ri \\ (1 - 16Ri)^{-0.75} & Ri < -0.1 \end{cases} \quad (34)$$

$$Ri = \frac{gz(T_a - T_m)}{u_z^2 T_a} \quad (35)$$

where g is the gravitational acceleration, and T_a and T_m are air and manure surface temperatures, respectively. The correction factor (l) is calculated as shown by Monteith and Unsworth (1990). l is the height of the internal boundary layer, that is, the distance from the slurry or soil surface to the point where the atmospheric NH₃ concentration equals the background concentration. The following approximate equation for l is used (van der Molen *et al.*, 1990a):

$$l \left(\ln \frac{l}{z_0} - 1 \right) = k^2 y \quad (36)$$

where y is the downwind distance from the border for the manure store.

The resistance of the laminar boundary layer r_b above manure or soiled surface is estimated using the empirical relationship by Thom (1972):

$$r_b = 6.2u_*^{-0.67}. \quad (37)$$

The resistance of the slurry surface layer r_c has to be estimated for different surface characteristics of the stored slurry (Olesen and Sommer, 1993).

If the store is covered by a roof, this will increase the NH_3 concentration in the atmosphere immediately above the manure surface, and reduce the concentration gradient across the NH_3 aqueous–gaseous interface. When the NH_3 concentration under the cover is in equilibrium with the gaseous NH_3 concentration at the top layer of the manure, no NH_3 is released from the NH_3 sources. Air movement under the cover is insignificant because the cover is airtight or quasi-airtight. Ammonia release from the manure is via diffusion mass transfer. In this situation, NH_3 emission is mainly determined by the resistance (permeation or leakage) of the cover.

D. SIMPLE GRADIENT APPROACH

The resistance model approach can be used when calculating the NH_3 emission from all sources. For some systems where we have insufficient knowledge about the transport processes or not enough input data are available one may use a simple gradient technique as presented by Sherlock *et al.* (1995). The rate of NH_3 emission from a liquid surface with TAN is given by:

$$F_{\text{NH}_3} = K_t \times u \times (\text{NH}_{3,\text{G}} - \text{NH}_{3,\text{A}}) \quad (38)$$

where F is the flux of NH_3 ($\text{g NH}_3\text{-N m}^{-2} \text{ s}^{-1}$), $\text{NH}_{3,\text{G}}$ is the concentration of atmospheric NH_3 in equilibrium with NH_4^+ in the liquid, and $\text{NH}_{3,\text{A}}$ is the NH_3 concentration of the free atmosphere ($\text{g NH}_3\text{-N m}^{-3}$). K_t is a transfer coefficient, $\text{NH}_{3,\text{G}}$ concentration ($\text{g NH}_3\text{-N m}^{-3}$) is calculated with Eq. (6). The ambient concentration of $\text{NH}_{3,\text{A}}$ is considered to be much lower (>100

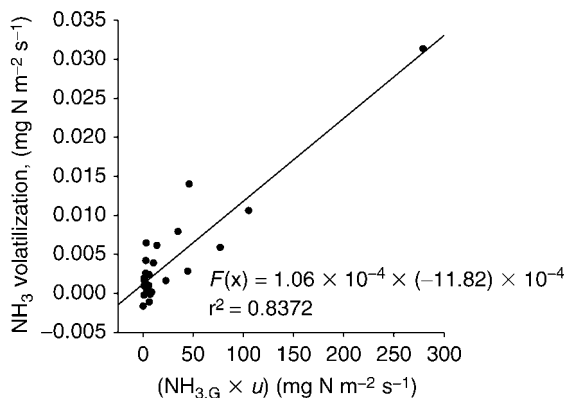


Figure 6 The relation between emission and NH₃ in the air in equilibrium with NH₃ in the slurry-soil surface (adapted from Sommer *et al.*, 2001).

times) than the concentration of NH_{3,G} in equilibrium with dissolved NH_{3,L}, therefore, most researchers decide to omit NH_{3,A} from the calculation. Tests have shown that the relation of NH₃ emission to NH_{3,G} × u is linear (see Fig. 6; Sherlock *et al.*, 1995, 2002; Sommer *et al.*, 2001). The coefficient K_1 is determined empirically and is affected by the height at which wind speed has been measured. In the study of Sherlock *et al.* (1995) with wind speed measured at 1.2 m, the slope was between 0.63×10^{-4} and 0.75×10^{-4} and significantly lower than determined when wind speed was measured at 0.1 m height (Sommer *et al.*, 2001), because wind speed is lower at 0.1 m than at 1.2 m.

V. MANURE CHEMISTRY

The source of NH₃ emission from livestock production is TAN [Eqs. (4)–(6)]. The source of TAN in manure from pigs, cattle, and sheep is mainly the organic component urea in urine (Elzing and Monteny, 1997; Oenema *et al.*, 2001). In cattle and pig production, urine is therefore recognized as being an important input variable for calculating NH₃ emission from animal housing, manure storage, the application of animal manure, and from pastures grazed by livestock.

During storage in animal housing, storage facilities, and beef feedlots, the amount of TAN in manure may vary due to transformation of N between organic N and TAN. There is no TAN in fresh feces or urine. The organic N

excreted has to be transformed to TAN by enzymes or through metabolism by microorganisms. The amount of TAN in this pool is also affected by production and emission of reduced and oxidized N and transformation of N between the organic and the inorganic pool of N in manure.

A. EXCRETION

1. Ruminants

Under most circumstances, the production level achieved by ruminants is determined by the amount of metabolizable energy from the feed ingested. Energy is normally limiting ruminant productivity and hence the retention of N by the ruminant. Variation in the amount of protein offered compared to the amount of protein needed for the production levels achieved, therefore, leads to large changes in the total amount of N excreted. Besides this total amount, also the partition of N excretion with urine and feces is strongly affected by the type of diet offered. In this respect, rumen functioning in particular is important.

Oenema *et al.* (2001) and Moss *et al.* (2000) have presented a comprehensive review of microbial transformation of N and biomass by ruminants. The rumen functioning control the amount of metabolizable energy and protein the ruminant may derive from the feed, and therefore, the fate of the N ingested. Urea is produced by the liver from NH_3 circulating in blood, formed with either protein fermentation in the rumen or from metabolizable protein not retained and oxidized by the ruminant. A surplus of fermentable crude protein (including feed NH_3) compared to fermentable carbohydrates in the rumen leads to an increase in the amount of NH_3 formed in the rumen and in the amount of NH_3 absorbed from the gastrointestinal tract to blood. More NH_3 in blood adds to urea excretion with urine. On the other hand, if the content of crude protein in feed is low compared to the content of fermentable carbohydrates the NH_3 concentrations in the rumen drop, urease activity of the rumen microbial population increases and substantial amounts of urea diffuse from blood to the rumen and thereby becomes an additional source of N for microbial protein synthesis next to ingested N. In the last decade, several modeling exercises have been published in which the factors controlling rumen fermentation and rumen N dynamics have been explored (Baldwin *et al.*, 1987; Danfaer, 1990; Dijkstra *et al.*, 1992) and reviewed (Bannink and de Visser, 1997; Offner and Sauvant, 2004). These studies on rumen functioning clearly indicate the complexity of the interactions between the amount of feed ingested and the type of carbohydrate and crude protein the feed is composed of (Dijkstra, 1993). Rumens functioning not only determine the type of nutrients absorbed from the gastrointestinal

tract, but it also determines the amount of fermentable organic matter flowing into the large intestine. Although the fermentation capacity of the large intestine seems limited in ruminants, still substantial amounts of material may become fermented, which leads to an increased synthesis of microbial N retained in feces instead of being excreted as urea with urine. Hence, also large intestinal fermentation may substantially affect the N dynamics in the ruminant and may cause shifts of up to 20% in the amount of N excreted with feces (Valk *et al.*, 1994). Typical feed ingredients stimulating fermentation in the large intestine are beet pulp and maize products. The use of different starch sources in ruminant diets may also lead to shifts in the amount of starch entering the large intestine. Mills *et al.* (1999) indicated that on average 6% of feed starch is fermented in the large intestine, but this may increase to 26% depending on diets or pretreatment of feed (Knowlton *et al.*, 1998). Therefore, digestion in the large intestine should not be neglected as a determinant for N in excretion.

Besides the balance between rumen fermentable carbohydrates and protein, also the balance between the amount of protein absorbed by the intestine (microbial as well as rumen unfermented protein) and the amount of metabolizable energy is important. An excess of metabolizable protein compared to the amount needed for the level of production achieved will reduce the efficiency of utilization by the ruminant, and more N will end up as urea in urine.

Summarizing, a low excretion of urea can be achieved by feeding high-quality diets (supporting ruminant production and N retention) that are low in crude protein (reducing N excretion). For example, a silage-based diet with low content of rumen degradable protein reduced urea N to 4.9 g kg⁻¹ urea in urine of lactating dairy cows compared to 8.4 g kg⁻¹ obtained with a diet with a high content of rumen degradable protein. Consequently, measured NH₃ emission was reduced by 39% (Smits *et al.*, 1995). Including forages containing condensed tannins or polyphenols in the diet will protect a proportion of the dietary protein from rumen degradation, thus allowing more extensive protein digestion in the abomasums and small intestine and greater subsequent absorption of amino acids without adversely affecting feed consumption or digestion (Min *et al.*, 2003). An additional effect is the decrease of the proportion of N excreted as urine compared to that excreted with feces (Misselbrook *et al.*, 2005a; Powell *et al.*, 1994).

Retention of ingested N being retained in milk varies from ~20% (e.g., mainly grass based diets) to ~30% (e.g., mainly maize and concentrate based diets), and in consequence, from ~70 to ~80% of the N is excreted with urine and feces. From 20 to >50% of the total amount of N excreted is collected in feces and 50–80% in urine. At surplus intake of digestible protein more N is excreted and most ends up as urea in urine. De Boer *et al.* (2002) found that urea concentrations in cattle urine could be predicted with reasonable

accuracy from existing models, which predict urine volume and urinary N excretion (Bannink *et al.*, 1999; Tamminga *et al.*, 1994) and an empirical relationship between urinary N and urinary urea concentrations. Besides the amount of urea excreted also urine volume strongly determines urea concentrations in urine and hence of NH_3 concentrations in urine puddles. Furthermore, urine volume and fecal water contribute to manure volume to a similar extent under normal conditions. This means that changes in urine volume or in the dry matter content of feces both have a large effect on TAN concentrations in manure. There are few options for changing pH of urine and manure from ruminants through change in diets (Oenema *et al.*, 2001).

2. Pigs

In comparison to ruminant feeding, the range in type and quality of fed ingredients used is narrow. Excretion of N in urine and feces from pigs depends on composition of the diet and the physiological status or the growth stage of the animals. The upper limit of protein deposition is affected by physiological status, age, gender, and energy supply. For pigs the excretion of N varies between the different stages of the reproductive cycle for sows and life cycles for pigs for slaughter. The amount of N excreted may be 18% of feed N intake for piglets (0–7.5 kg) and 36% for growing pigs (Fernández *et al.*, 1999). Nitrogen excreted in the feces amounts to 17% of intake and corresponds largely to the undigested protein fractions. Digested proteins are absorbed as amino acids and are used for deposition in body protein. Because a surplus of absorbed amino acids will not be stored for later use (Moughan, 1993), this surplus will be oxidized and the N is excreted mainly as urea with urine.

When the amino acids absorbed are unbalanced in relation to the requirement for synthesis of body protein, most of the unbalanced amino acids will be oxidized as well. Similar to the excess of total amino acid supply, the N from these unbalanced amino acids will be excreted as urea (Fernández *et al.*, 1999). Nitrogen utilization has been improved by ensuring an adequate protein and amino acid supply over time according to the growth potential and physiological status of the animal and by improving dietary amino acid balance and consequently reducing the protein content of the diet (Henry and Dourmad, 1993). By supplementing feed with synthetic amino acids N, the protein content of the feed may be reduced, leading to a reduction in N excretion up to 35% without affecting daily weight gain, feed efficiency, and carcass composition (Dourmad *et al.*, 1993; Noblet *et al.*, 1987). There is a limit, however, to the reduction of dietary protein contents because a too large reduction may cause a deficiency of nonessential amino

acids (Wang and Fuller, 1989). Improving protein quality by adding essential amino acids to the feed is a powerful measure to reduce N excretion with urine without compromising production results.

Changing feeding strategy is a most efficient method for reducing excretion of N. As fattening pigs mature, the need for N in relation to energy demand gradually decreases. Consequently if farmers feed a constant protein concentration the amount of N excreted will increase with increasing weight of the animal. Reducing the ratio of protein to energy in the feeding ration (phase feeding) will reduce excretion of N at increasing age of the finishing pig. Using different diets during the growing and feeding periods may reduce N excretion by 8% compared with using the same diet during the whole growth period (Latimier and Dourmad, 1993). Nitrogen excretion may be reduced further by multiphase feeding, mixing two diets with appropriate proportions of protein, and amino acids during the growth period (Bourdon *et al.*, 1997), thereby, reducing excretion to 50% of the N intake (Bourdon *et al.*, 1997; Chung and Baker, 1992).

Knowing the biorhythm in pig metabolism (Koopmans *et al.*, 2005) may contribute to a reduction in N excretion. An increased postprandial efficiency of protein metabolism is achieved in the morning compared to the evening, and this would imply that a lower protein content in the evening diet compared to the morning diet would give the same production results. Besides protein digestion and amino acid supply to the pig and the above feeding strategies involved with protein nutrition, making use of the fermentative capacity of the large intestine is also a potential measure to cause a shift in N excretion from urea with urine to microbial N with feces. Bakker (1996) clearly demonstrated the large fermentative capacity of the large intestine. Van der Meulen *et al.* (1997) established that replacement of 65% of cornstarch for potato starch resulted in an increase of the amounts of urea N recycled from blood urea to the intestine of 21–124% of the NH₃-N absorbed from the entire gastrointestinal tract. Reasonable relationships were established between the amount of fermentable (so-called nonstarch) polysaccharides included in the diet and the ratio of urine N to fecal N. Increasing the content from 100 to 650 g kg⁻¹ of dietary dry matter resulted in a strong curvilinear reduction of this ratio from 4 to 1 (Jongbloed, personal communication). In particular the increase from 100 to 200 g kg⁻¹ dry matter resulted in a strong reduction (50%) of this ratio. The lower value than one for this ratio of urine to fecal N corresponds to the value established with 65% of readily degradable raw potato starch included in the diet (Bakker *et al.*, 1996; van der Meulen *et al.*, 1997).

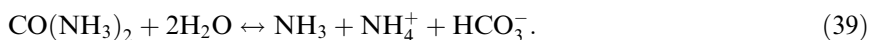
An additional effect of reducing N excretion by giving the pigs a low-protein and high-fiber diet is that the pH of slurry is reduced (van der Peet-Schwering *et al.*, 1999). Small fractions of the volatile fatty acids (VFS) formed in the intestine is excreted in feces and reduce pH of feces

and fresh manure. Besides the inclusion of fermentable carbohydrates in the diet also a reduction of urine pH will reduce NH_3 emission (Canh *et al.*, 1998b). Low urine pH can be achieved by adding salts to the diet that cause a reduction of the charge of cations relative to the charge of anions in the diet.

Most of the nutritional factors discussed have an additive effect on TAN in manure and hence on NH_3 emission. The amount of electrolytes excreted with urine strongly determines urine volume, and consequently the TAN concentrations in urine and manure. Although feces contributes much less to manure volume than with cattle, much variation may occur in the dry matter content of feces, which may be reduced by 60% (Cahn *et al.*, 1997) f. ex. if sugar beet pulp is replaced by tapioca in the diet. Furthermore, Aarnink *et al.* (1992) indicate an increase in dry matter content of more than 0.1% per kg increase of live weight. Although such changes have a moderate effect on manure volume, it does alter the consistency of feces and hence NH_3 emission rates. The composition of pig slurry may be estimated using the algorithms of Aarnink *et al.* (1992).

B. UREA TRANSFORMATION TO AMMONIUM

The TAN in pig, cattle, or sheep manure originates mainly from the hydrolysis of the urea in urine by the enzyme urease. Urea is a diamide, which is transformed by urease to NH_3 , NH_4^+ , and bicarbonate (HCO_3^-):



The feces excreted by livestock contain bacteria producing urease, therefore, urease is abundant on the housing floors and soils in beef feedlots and exercise areas (Elzing and Monteny, 1997; Whitehead, 1990). In livestock houses, the abundance of urease is positively related to surface roughness, and urease activity on floors is usually greater (up to a factor 10) than the urease activity of slurry (Braam and Swierstra, 1999; Elzing and Monteny, 1997; Muck, 1982). Only the reduction in urease activity due to the cleaning of very smooth coated floors has been shown to affect NH_3 emission from livestock buildings (Braam and Swierstra, 1999).

Hydrolysis of urea is affected by pH (Muck, 1982; Ouyang *et al.*, 1998) and optimum pH for urease activity has been reported to range from pH 6–9. Animal manure pH is buffered to between 7 and 8.4; therefore, hydrolyses of urea will not be greatly influenced by pH in manure that has not been treated with acids and bases. It is in general found that urease activity on floors is very persistent and only aggressive cleansing (e.g., with strong acids) can reduce urease activity.

The urease activity is affected by temperature, and the activity is low at temperatures below 5–10°C and at temperatures above 60°C (Moyo *et al.*, 1989; Sahrawat, 1984; Xu *et al.*, 1993). In models the urease activity has been depicted as being exponentially related to temperature (Braam *et al.*, 1997). In livestock buildings increase in the rate of urease activity is slow below 5–10°C, and its development increases exponentially above 10°C (Braam *et al.*, 1997; Le Cadre, 2004). Thus,

$$K_{UA}(T) = K_{UA,T_{ref}} \times Q_{10}^{\frac{T-T_{ref}}{10}} \quad (40)$$

where $K_{UA}(T)$ is the urease constant (kg N m⁻³ of urine per second), $K_{UA,T_{ref}}$ is the urease constant at the reference temperature (T_{ref} , 25°C), T (°C) is the temperature, and the value of Q_T is set to 2.

At urea concentrations higher than 3 M, hydrolysis may be inhibited (Rachhpal-Singh and Nye, 1986), but at concentrations up to this threshold hydrolysis will increase with increasing urea concentration on the floor. Monteny, G. J. (personal communication) proposes the following equation relating urease activity to urea–N concentration of the manure:

$$K_{UA} = 2.7 \times 10^{-3} \times (\text{urea-N}). \quad (41)$$

Thus, in practice, only temperature and urea concentrations may significantly affect hydrolysis rate to a degree that will rate control NH₃ emission (Braam *et al.*, 1997; Monteny *et al.*, 1998), meaning that extreme measures, such as rinsing with strong acid or formaldehyde, are required in order to achieve a substantial reduction.

C. TRANSFORMATION OF N BETWEEN INORGANIC AND ORGANIC POOLS

Immobilization of inorganic N into organically bound N is a microbial process, which depends on the C:N ratio of degradable organic compounds. When the C:N ratio of the degradable compounds in animal manure is high, inorganic N from the manure is immobilized into microbial biomass. Conversely, when the C:N ratio of the degradable compounds in animal manure is low, organically bound N is transformed (mineralized) into inorganic N. Hence, immobilization decreases the amount of TAN, while mineralization increases the amount of TAN, the balance of which depends on the C:N ratio of degradable C in the animal manure (Kirchmann and Witter, 1989). Cattle slurry has a greater fraction of poorly degradable C than pig slurry (Kirchmann, 1991).

Typically, the C:N ratio of feces is 20 and that of urine is in the range 2–5. The C:N ratio of urine is low and rapidly decreases further following excretion because of the hydrolysis of the easily degradable compounds (see earlier). Slurry mixtures have C:N ratios in the range from 4 for pig slurries to 10 for cattle slurries (Chadwick *et al.*, 2000). The concentration of N in feces of cattle is usually in the range 20–40 g kg⁻¹ DM⁻¹, while the N concentration in urine may range from 1 to 20 g liter⁻¹, depending on the protein content of the animal feed and production level (Bussink and Oenema, 1998). Roughly half of the N in feces is undigested and nonabsorbed dietary N, while the other half is endogenous, resulting from enzymes and mucus excreted into the digestive tract. The undigested dietary N in feces is poorly degradable, unlike the endogenous N.

In general, there is no immobilization of N in slurry mixtures stored in an anaerobic environment, because the C:N ratio of the easily degradable compounds is low (<15) (Kirchmann and Witter, 1989; Thomsen, 2000). The addition of straw and other bedding material with a high C:N ratio increases the amount of degradable C and induces immobilization. As a result, farmyard manure (i.e., a mixture of mainly feces and bedding material with a small amount of urine added) typically has a high C:N ratio and low TAN (Külling *et al.*, 2003). Kirchmann and Witter (1989) estimated an immobilization potential of 11.2 mg N g⁻¹ straw at a C:N ratio between 18 and 24, and 2.2 mg N g⁻¹ straw at a ratio between 24 and 36. They cited Richards and Norman (1931) as having reported a similar immobilization potential of straw.

Because immobilization of inorganic N in animal manure is uncommon, except for bedding material amended farmyard manure, there are no algorithms developed specifically for immobilization in animal manure, according to our knowledge. However, for modeling immobilization in animal manure, use can be made of the algorithms developed for immobilization in soil.

In slurry, transformation of organic N to inorganic N (mineralization) appears to occur during storage (Sørensen, 1998; Zhang and Day, 1996). During in-house storage, most of the digestible compounds containing N are transformed and about 10% of the organic N is mineralized (Zhang and Day, 1996). During outside storage of slurry, little N is mineralized and it is assumed that about 5% of the organic N is transformed to inorganic N during 6–9 month storage (Poulsen *et al.*, 2001). Few studies have completely quantified the anaerobic transformation of N in slurry stores, but the degradation is closely linked to transformation of C, and the models of anaerobic degradation of biomass may be used to calculate the N transformation (Cobb and Hill, 1993).

D. NITRIFICATION AND DENITRIFICATION

Nitrification is the oxidation of TAN (NH₄⁺ or NH₃) into nitrite (NO₂⁻) and then into NO₃⁻ by predominantly autotrophic microorganisms (Nitrobacteriaceae). The first step, the oxidation of TAN into NO₂⁻, is conducted by the so-called NH₃ oxidizers or primary nitrifiers, whereas the second step is carried out by NO₂⁻ oxidizers or secondary nitrifiers. *Nitrosomonas europaea* is the best studied NH₃ oxidizer, while *Nitrobacter winogradskyi* is one of the most common NO₂⁻ oxidizer. The Nitrobacteriaceae are aerobes and many are obligate autotrophs, that is, they require oxygen (O₂) and the energy required for growth originates from nitrification. However, NH₄⁺, NH₃, and NO₂ are not very effective energy sources, making the Nitrobacteriaceae slow growers. They are also highly sensitive to pH; nitrification is negligible at pH values less than ~4 and increases linearly as pH increases from 4 to 6 (Winter and Eiland, 1996). Currently, there is increased interest in the process of nitrification because of the possible release of the intermediate N₂O during NH₃ oxidation and NO₂⁻ oxidation (Wrage *et al.*, 2001). Nitrous oxide is a potent greenhouse gas and nitrification of TAN in animal manure is a possible important source (Oenema *et al.*, 2001).

Because feces and urine are highly anoxic upon excretion, nitrifying activity is absent. During storage of animal slurries, nitrifying activity develops only slowly at the interface of atmosphere and slurry (Fig. 3), because the diffusion of molecular O₂ into the slurry is slow (Petersen *et al.*, 1996), the biological demand by the host of competing microorganisms is large, and Nitrobacteriaceae are slow growers and thus have a competitive disadvantage. Surface drying may accelerate the creation of oxic conditions at the surface and therefore may induce nitrifying activity during long-term storage. However, the amount of TAN nitrified in slurries and liquid manures in lagoons and basins is usually very small. Also the release of N₂O from slurry during storage is small (Harper *et al.*, 2000; Külling *et al.*, 2003; Oenema 1993; Velthof *et al.*, 2005).

In bedding-material-amended animal manure in deep litter stables, feedlots, and in stacked farmyard manure heaps, significant nitrifying activity can be developed during storage. Here, the nitrifying activity results from the much greater aeration of the manure in the surface layer compared with slurry, because the litter-amended manure is rather dry, thus allowing molecular O₂ to diffuse more easily into the manure, while the added straw litter may also serve as a conduit for molecular O₂ and the oxygenation of the manure. As a result, measurable quantities of NO₂⁻ and NO₃⁻ can be found in the surface layers, and also significant emissions of N₂O have been measured from dung heaps and deep litter stables (Berges and Crutzen, 1996; Chadwick, 2005; Groenestein and Van Faassen, 1996; Petersen *et al.*, 1998a; Sibbesen and Lind, 1993).

Modeling of nitrification is based either on a mechanistic description of the growth and development of nitrifying populations (Li *et al.*, 1992) or simply as a substrate-dependent process using first-order kinetics (Gilmour, 1984; Grant, 1994; Malhi and McGill, 1982). The microbial growth models consider the dynamics of the nitrifying organisms responsible for the nitrifying activity. The simplified process models are easier to use and do not consider microbial processes and gaseous diffusion. In these simplified models, nitrification rate $[d(\text{TAN})/dt]$ is described as an empirical function of substrate concentration ($[\text{TAN}]$), oxygen partial pressure ($p\text{O}_2$), temperature (T), and pH according to

$$d(\text{TAN})/dt = k_1 \cdot f(\text{TAN}) \cdot f(p\text{O}_2) \cdot f(T) \cdot f(\text{pH}) \quad (42)$$

where k_1 is the first-order nitrification coefficient under optimal conditions, and $f(\text{TAN}) = [\text{TAN}]$. Sometimes, nitrifying activity is related to TAN concentration via a Michaelis-Menten type relationship, that is, $f(\text{TAN}) = [\text{TAN}]/(k_2 + [\text{TAN}])$. In this case, TAN is limiting nitrifying activity (c.f. first-order process) at low TAN concentration and TAN is not limiting nitrifying activity (zero-order) at high concentration. Constant k_2 is the Michaelis-Menten half-saturation constant, or the TAN concentration at which $f(\text{TAN}) = 0.5$. It should be noted that the meaning of k_1 changes to "potential nitrification activity," when a Michaelis-Menten type of relationship is used for substrate dependence.

A complex part of the model involves the calculation of the dependence on $p\text{O}_2$. Manure heaps and deep-litter in animal houses usually have a depth-gradient for porosity, air permeability and temperature, and thereby also for transport characteristics (diffusivity), O_2 consumption, and thermal conductivity into the manure. Van Ginkel (1996) derived a detailed mechanistic model of the temperature and $p\text{O}_2$ in a manure heap, and showed that the physical, chemical, and biological processes are mutually dependent. The moisture content is a critical factor for the O_2 diffusivity and $f(p\text{O}_2)$ is sometimes related to the water-filled pore space (WFPS), using an empirical equation of the form $f(p\text{O}_2) = \{\sin(\pi \times \text{WFPS}^a)^b\}$, where a and b are shape parameters. Hence, the reduction function $f(p\text{O}_2) = 0$ when WFPS is 0 and 100%, and $f(p\text{O}_2) = 1$ somewhere in between (usually at WFPS $\sim 60\%$), depending on the shape parameters a and b .

Like most biological processes, nitrifying activity generally increases exponentially with increasing temperature, until a certain temperature after which the activity decreases with increasing temperature (e.g., composting manure heaps). According to Arrhenius' law, the reduction function for temperature can be described by

$$f(T) = \exp \left[\frac{K_A(T - T_{\text{ref}})}{(T_{\text{ref}} \cdot T)} \right] \quad (43)$$

where T is temperature, T_{ref} the reference temperature where $f(T) = 1$, and K_A is a coefficient characteristic for the environment.

Summarizing, ammonium oxidizers consume TAN and thereby may potentially lower NH₃ volatilization. In slurry-based housing systems and in lagoons and slurry storage basins, nitrifying activity is usually low and probably has only a minor effect on total NH₃ volatilization losses. In feedlots, deep litter stables and manure heaps, though, nitrifying activity develops in surface layers and significant amounts of TAN can be transformed into NO₂⁻ and NO₃⁻, thereby reducing the potential for NH₃ volatilization losses.

E. pH BUFFER SYSTEM

Manure proton concentration [H⁺] affects the release of NH₃ to a great extent [Eqs. (4)–(6)]. Therefore, the buffer systems controlling [H⁺] in the surface liquid layers of the emitting sources should be known when developing models of NH₃ emission.

It has been shown that the main buffer components in animal manure controlling [H⁺] is total inorganic C (TIC = CO₂ + HCO₃⁻ + H₂CO₃), TAN and VFA = C₂–C₅ acids (Sommer and Husted, 1995a; Vavilin *et al.*, 1998). Sommer and Husted (1995b) showed that pH can be calculated with a simple model based on the fact that the charge of the liquid should be zero and including calculations of the equilibrium concentrations of species of NH₃/NH₄⁺ [Eq. (4)] and of the following reactions:



where HAc is acetic acid representing the VFA in the manure.

Hydrolysis of urea produces a mixture of NH₃, NH₄⁺, HCO₃⁻, and CO₃²⁻ and this may increase pH, because NH₃ and CO₃²⁻ are bases (pK_a = 9.48 for NH₃/NH₄⁺ and pK_a = 10.4 for HCO₃⁻/CO₃²⁻). Therefore, the pH at the site

of excretion will increase initially due to the formation of bases in the fresh urine on solid floors, slurry in channels and in deep litter (Henriksen *et al.*, 2000a).

In slurry the concentration of TAN may initially be larger than the concentration of TIC, because hydrolysis of urea produces 2 mol TAN per mol TIC (Sommer and Husted, 1995a). In contrast TIC may be larger than TAN in the bulk of a stored slurry, because TIC is produced during anaerobic fermentation of organic material. At the surface, CO₂ is released more readily than NH₃ due to the lower solubility of CO₂ than that of NH₃. The greater loss of TIC than of TAN will increase pH [see Eq. (4) and TIC equations]. Without the balancing effect of TIC emission, NH₃ emission would cause a reduction in pH and thereby cause a reduction in NH₃ emission. These effects were shown in a study of the change in buffer components and pH in slurry stored in thin layers in Petri dishes (Sommer and Sherlock, 1996). There was a great increase in slurry pH over the first 8 h due to the release of CO₂, in slurry with the initial TIC > TAN; pH then increased steadily but slowly from 8 to 96 h. When the initial TIC was < TAN, the pH declined or did not change after 20-h incubation. The initial pH elevation rate increased with temperature and initial concentration of TIC.

Calculation with a pH buffer model indicated that the NH_{3,G} partial pressure in equilibrium with the slurry increased and pH decreased at increasing temperature if gases could not exchange between the slurry and the atmosphere (Sommer and Sherlock, 1996). The differential release of NH₃ and CO₂ from a slurry surface will be affected by ventilation in the animal houses, and a sudden reduction in pressure due to increased ventilation will cause an immediate increase in emission of CO₂ and an increased emission of NH₃ following the increase in CO₂ emission (Ni *et al.*, 2000).

Oxic degradation of organic material will reduce the content of acids in solution and thereby increase pH. In contrast anoxic processes will contribute to the formation of organic acids and thereby reduce pH (Fig. 7). The pH of manure will therefore differ between solid manure through which air is moving and anaerobic slurry or compact solid manure with no airflow through the bulk of the stored manure.

The surface of slurry in contact with oxygen in the air may have a smaller concentration of VFA than the bulk of slurry because the organic material is transformed to CO₂ through aerobic processes whereas the organic material in the bulk of the stored slurry is transformed to VFA and subsequently to methane (CH₄) and CO₂ (Møller *et al.*, 2004; Fig. 8). Thus, the pH in the surface of stored slurry may be much higher than pH in the bulk of slurry (Olesen and Sommer, 1993; Fig. 9).

In the bulk of the stored slurry the environment is predominantly anaerobic and organic material is degraded to volatile organic acids

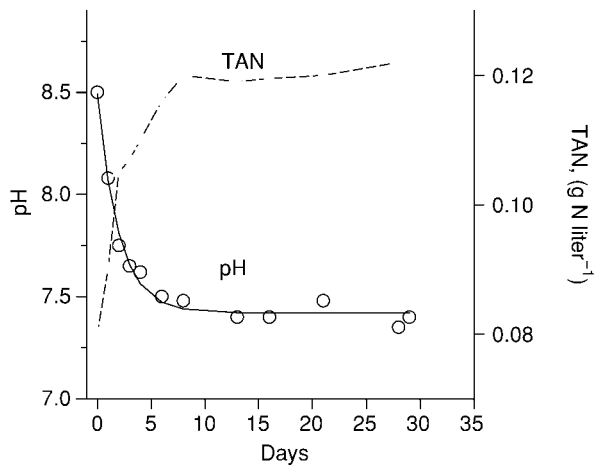


Figure 7 Changes in pH and total ammoniacal ammonium content (TAN = NH₃ + NH₄⁺) of newly mixed slurry (From Husted, 1992).

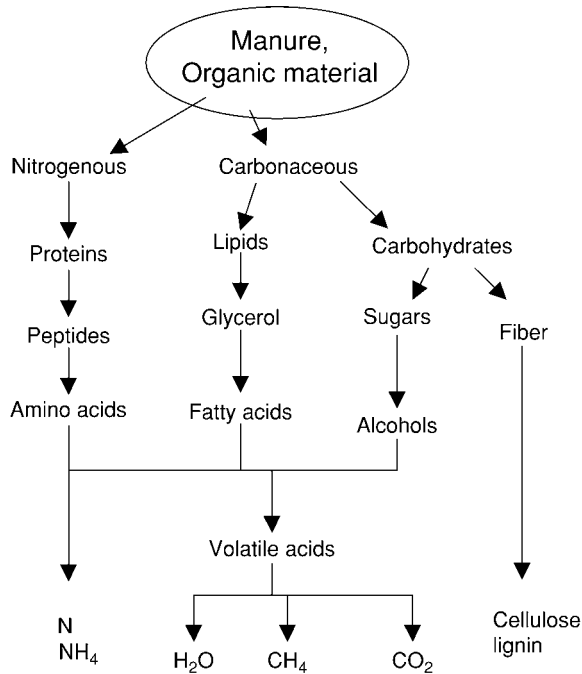


Figure 8 Major pathways for breakdown of feces (after Merkel, 1981; slightly modified).

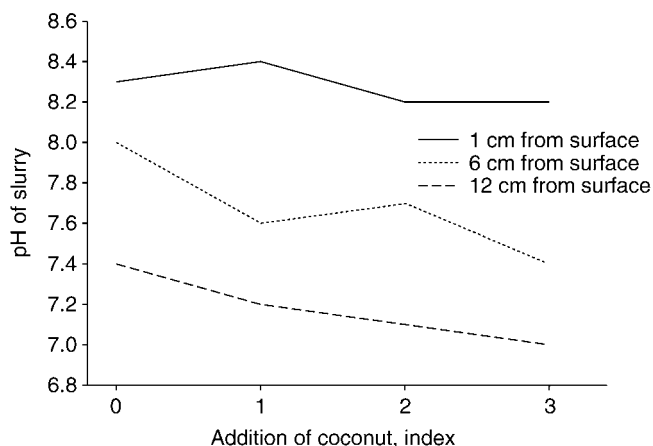


Figure 9 Slurry pH as affected by distance to surface of stored slurry and addition of digestible carbohydrates (index 0 is no coconut fat and 1–3 is increasing addition of coconut fat) to feed given to pigs (adapted from Canh *et al.*, 1998b).

(VFA = C_1 – C_5), which is the substrate for methanogenesis (Fig. 8). The first step in the processes is hydrolysis of the biomass to dissolved biopolymers (fat, cellulose, protein, lignin) a process catalyzed by exoenzymes. The biopolymers are transformed by bacteria into organic acids, hydrogen, CO_2 , and water (Acidogenesis), and the longer-chained organic acids are oxidized producing acetic acid, CO_2 , hydrogen, and water (Acetogenesis). The content of organic acid is reduced in the methanogenic step by transformation to CH_4 and CO_2 (Aceticlastic step).

These processes are related to feed intake, for example, a large intake of fiber will increase the VFA concentration in the feces and thereby reduce pH (Imoto and Namioka, 1978). Furthermore, a high NH_3 concentration and a high pH (interacting with NH_3) may inhibit methanogenesis and cause accumulation of VFA (Angelidaki *et al.*, 1993). High loading rates or sudden changes in loading rates of biomass in relation to the amount of slurry stored may also cause an increase in VFA due to a reduction in CH_4 production (Hill *et al.*, 2001). Further degradation of VFA occurs due to production of CH_4 decreasing with decreasing temperature and VFA therefore accumulates at temperatures below 10–20°C, causing a reduction in pH (Fig. 10).

Models have been developed that predict VFA and CH_4 production through anaerobic degradation (Fermentation) of organic industrial waste at temperatures above 50°C (Angelidaki *et al.*, 1993), at 6°C (Vavilin *et al.*, 1998), and at a range from 10 to 70°C (Hill *et al.*, 2001).

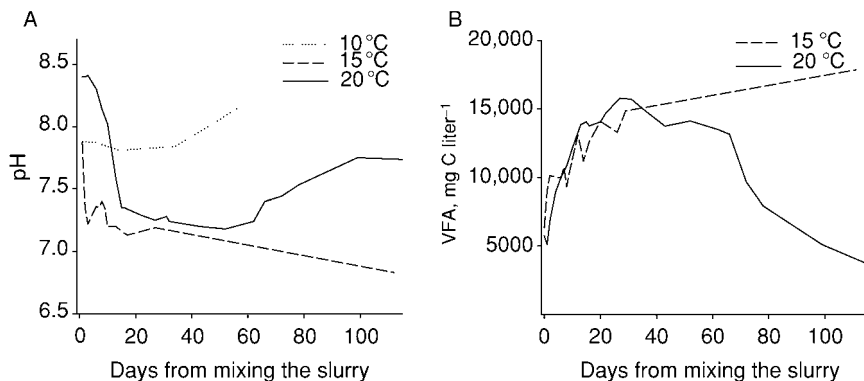


Figure 10 Change in pH in pig slurry stored at 10, 15, and 20°C (A) and VFA in pig slurry stored at 15 and 20°C (B) after mixing (Møller *et al.*, 2004; Sommer *et al.*, 2005).

Increasing or decreasing ionic species in the urine or slurry will affect the pH, because the electric charge of the solution has to be neutral (Sommer and Husted, 1995b). At present soybeans in the diet are supplying most of the crude proteins needed by the pigs, and soybean contains high concentrations of K⁺, which when excreted will increase the pH of urine and slurry. Reducing the soybean concentration in the diet and supplementing with amino acids will reduce the K⁺ concentration and increase H⁺ concentration (reduce pH) according to Eq. (47).

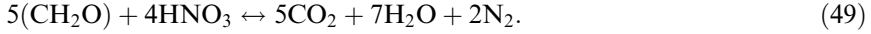
$$Z_{\text{system}} = ([\text{Na}^+] + [\text{NH}_4^+] + [\text{K}^+] + 2 \times [\text{Ca}^{2+}] + 2 \times [\text{Mg}^{2+}] + [\text{H}^+]) - ([\text{HCO}_3^-] + 2 \times [\text{CO}_3^{2-}] + [\text{Ac}^-] + [\text{Cl}^-] + [\text{OH}^-]) \quad (47)$$

where Z_{system} is the charge of the solution (Sommer and Husted, 1995b). Thus, for pig urine and slurry and for cattle urine it has been shown that pH declines when cationic species of the feed is reduced; that is, for pig slurry a reduction of more than 1 pH unit has been observed within the range of traditional diets with and without addition of amino acids and reduction of soybean (Bannink and van Vuuren, 1998; Canh *et al.*, 1998a, cited in Oenema *et al.*, 2001; Portejoie *et al.*, 2004).

Nitrification and denitrification in the surface of slurry or in the liquid phase of stored solid manure may also affect pH. Therefore, nitrification may affect NH₃ emission through reduction in TAN and by reducing pH, as nitrification of 1 mol NH₄⁺ produces 2 mol H⁺, according to the following equation:



and denitrification may affect pH according to the following equation (Petersen *et al.*, 1996):



In urine deposited on concrete floors (with high hydrolysis activity) the pH increased exponentially initially to a level 1 pH unit higher than the original urine pH (~ 8.5) for urine on clean or scraped floors (Monteny, 2000); for urine deposited on slurry in the slurry channel, this increase is ~ 1.3 pH unit higher than the slurry pH (~ 7.5). It is likely that urine pH is buffered by the material on the surface area where it is deposited, and that difference in emission of CO_2 and NH_3 emission will affect the pattern of the change in pH over time. In line with this, pH in urine deposited on floors fouled with feces shows the same increase as for clean floors but at a much lower level (fecal pH is lower than the pH of concrete).

F. CATION EXCHANGE CAPACITY OF SOLID MATTER IN MANURE

The dry matter fraction in slurry and of solid manure contains organic matter with functional groups that are weak acids (Bril and Salomons, 1990; Sommer and Husted, 1995a), so the organic material will be negatively charged at $\text{pH} > 7.5$, which is common in most slurries (see <http://www.alfam.dk/>). Henriksen *et al.* (2000b) found the adsorption capacity of manure DM was $1.4 \text{ mol kg}^{-1} \text{ DM}^{-1}$, which corresponds to the concentration of acid groups on DM in animal slurry (Sommer and Husted, 1995a). In comparison, soil organic matter may have an exchange capacity of about 2.50 mol kg^{-1} at pH 8 (Rhue and Mansell, 1988). More than 95% of the slurry TAN (Fig. 4) will be in the NH_4^+ form and can be exchanged using the slurry CEC. The slurry also contains high concentrations of the divalent cations Ca^{2+} and Mg^{2+} , which have a higher affinity for adsorption than NH_4^+ . Therefore, the exchange of NH_4^+ with slurry CEC can be defined using the Gapon equation (Russell, 1977):

$$\frac{(\text{NH}_4^+)}{\sqrt{(\text{Ca}^{2+})(\text{Mg}^{2+})}} = K_g \frac{\text{Ex} - \text{NH}_4^+}{\text{Ex} - (\text{Ca}^{2+} + \text{Mg}^{2+})} \quad (50)$$

where Ex-NH_4^+ and $\text{Ex-(Ca}^{2+} + \text{Mg}^{2+})$ are, respectively, the NH_4^+ and $\text{Ca}^{2+} + \text{Mg}^{2+}$ ions bound to the slurry CEC, and K_g is the Gapon coefficient. The consequence of the exchange processes is that dilution of the DM with rain or irrigation water will change the equilibrium and the divalent cations in solution will be exchanged with NH_4^+ (Chung and Zasoski, 1994). Conversely, if the solution is concentrated by water being removed due to drying, NH_4^+ will exchange with divalent cations of the DM. Thus, during a drying event, the concentration of NH_4^+ in solution will increase less than linearly with the evaporation of water.

VI. EMISSION FROM LIVESTOCK HOUSING

The emission of NH₃ from livestock housing in four European countries was examined in the mid-1990s (Groot Koerkamp *et al.*, 1998b). The results from that study indicate that emission differs widely between animal categories and housing systems. The source of this variation is discussed in the following sections and, when feasible, coefficients and algorithms that may encompass this variation are presented.

A. CATTLE HOUSING

1. Slatted Floor

a. Release and Transfer Ammonia emission from cattle on slatted floors varies between cattle categories due to differences in feeding and housing. Thus, dairy cows are given a greater percentage of N in their ration than are calves and beef cattle. Beef housing and most new dairy houses are naturally ventilated, although forced ventilation may have been more common in older dairy houses.

Approximately 40% of the NH₃ in a cubicle dairy cow house with slatted floors originates from slurry stored in the pit below the slatted floor, and the remainder is produced from urea deposited on the slats (Braam and Swierstra, 1999; Monteny, 2000). The emission from the floor is relatively constant, whereas the pit emission fluctuates depending on the temperature difference between the air inside the pit and that above the slats (Monteny, 2000). In periods with a positive temperature gradient (e.g., relatively warm pit air), the emission from the pit may account for over 75% of the total emission from the house due to convective air exchange between pit and the house, whereas pit emissions are as low as 20% in the situation of relatively cold air in the pit creating a stagnant layer of air in the pit and NH₃ is

transported by diffusion. The NH_3 concentration that builds up in the pit with cold air reduces the release of NH_3 from the slurry.

The emission is related to indoor and in consequence to the outdoor temperature. Thus, in summer in the Netherlands emission is higher than during winter (Kroodsma *et al.*, 1993), because at higher temperatures ventilation increases which increases the transfer coefficient and also slurry temperature increases which increases the concentration of $\text{NH}_{3,\text{L}}$ in manure. The emission is related to the soiling of the floor. Thus, in the United Kingdom, NH_3 emission in the summer was 56% of the emission during winter (Phillips *et al.*, 1998), because the animals only had access to part of the building in summer and only ~50% of the area soiled during winter was soiled during summer. In a situation of full occupation of the house, each part of the slatted floor is wetted by a freshly deposited urination on average once every 8 h during winter (Monteny, 2000). In the Netherlands, when animals leave the house for grazing during summer, no fresh urine is deposited in most of the day (only when the cows enter the house for being milked). However, ammonia emission from the urine remaining on the floor surface area continues for approximately 8 h, but the emission rate decreases exponentially with time (Kroodsma *et al.*, 1993).

b. Gross Emission Factors The loss of NH_3 from cattle housing systems with slatted floors in Denmark (Poulsen *et al.*, 2001) is estimated at about 8% of the total-N in the slurry. Estimated losses of NH_3 from dairy cattle housing systems with slatted floors in the Netherlands range from 2 to about 15% of the total-N in the cattle slurry (Monteny and Erisman, 1998). This wide range is caused by diet composition, the large difference in the areas of fouled floor between tie stalls and cubicle houses, and by the difference in housing period (i.e., cattle are housed for 180 day year⁻¹ in tie stalls and all year round in cubicle houses). In Monteny and Erisman (1998), an overview is presented of emissions from various types of dairy cow houses. In general, emissions from cubicle houses are between 20 and 45 g $\text{NH}_3\text{-N}$ cow⁻¹ day⁻¹, whereas emissions from dairy cows housed in tying stalls are less (5–21 g $\text{NH}_3\text{-N}$). This lesser emission from cows housed in tying stalls is directly related to the reduced floor area of on average 3.5 and 1 m², respectively for cubicle and tying stalls. As a rule of thumb, these emissions are equivalent to 10–15 g $\text{NH}_3\text{-N}$ m² and day (the area relates to floor and pit). The ranges indicated are mostly related to aspects such as diet and climatic conditions. When correcting for temperature and animal density, diets cause a range in the emission factor of 5–12% of the N excreted (or 10–23% of the TAN in slurry, assuming 50% of total N being in the form of TAN (Monteny, 2000). Depending on the diet, urinary N concentration in the urine may range from 3 to 12 g N liter⁻¹. Since one urination is found to cover 1 m² of slatted floor area, leaving a layer of 0.5 mm of urine, 80% of each urine deposition (on average 4 liter urine per

Table IV
Ammonia Emission Factors for Cattle Buildings (Amon *et al.* (2001); Groot Koerkamp *et al.* (1998b); Kroodsmas *et al.* (1993); Rom and Henriksen (2000))

Building design	Pen design	Emission factor (% of total-N)	Emission factor (kg NH ₃ -N per kg TAN ^a)
Tie stalls	Slurry	3	0.6
Cubicle	Partly slatted floor, 0.4 m deep slurry channel	6	0.12
Cubicle	Partly slatted floor, 1.2 m deep slurry channel	8	0.17
Solid floor	Deep litter	6	0.12

^aTAN = NH₃ + NH₄⁺.

urination) flows through the slots to the slurry pit. The remaining 1.5–6 g urea N m⁻² of floor area is converted to TAN and is potentially available for emission (depending on pH, temperature, and air velocity). An estimate of average NH₃ emission factors is given in Table IV.

c. Reduction Measures One of the most important factors controlling NH₃ emissions is the surface area of soiled surfaces (Monteny and Erisman, 1998; Sommer and Hutchings, 1995; Voorburg and Kroodsmas 1992). This may be achieved either by reducing the area where the animals excrete or by cleaning the floor soiled by excreta. The efficiency of different technologies is given in the following.

Monteny and Erisman (1998) found that NH₃ emissions from cows in tie stall were 35% less than those kept in cubicles, mainly caused by a reduction in area of floor covered by feces and urine and slurry pit surfaces.

Reduction in the emission of NH₃ might be achieved by the rapid removal of urine and feces from the livestock buildings and their containment in covered stores. For a solid concrete 3% sloping floor, the rate of NH₃ volatilization relates to the total urinary N retained on the floor, and NH₃ emission is a function of the production of NH₃ in solution, that is, hydrolysis of urea.

Scraping a nonsloping concrete floor will have little effect on the NH₃ because a thin layer of liquid with TAN is retained by the floor, which will be a significant source of NH₃ (Braam *et al.*, 1997; Oosthoek *et al.*, 1991). If the floor is smooth, scraping may reduce emission by up to 30%, but to the detriment of animal welfare (Braam and Swierstra, 1999; Oosthoek *et al.*, 1991). Scraping a sloping floor with gutters at both sides or in the middle of the gangway may reduce emission by about 21% with scraping every 12 h

(Braam *et al.*, 1997). Frequently scraping a grooved solid floor with or without gutters for urine outlet may reduce emissions by about 50%.

Scraping an inclining solid floor followed by water spraying may reduce emission by 65% (Braam *et al.*, 1997; Swierstra and Braam, 1999; Swierstra *et al.*, 1995). Thus, it is the combination of cleaning the floor with a scraper and draining the urine freely to a gutter that reduces the NH_3 release from the floor and reduces NH_3 emission from the animal building. Scraping a slatted floor and spraying the floor with formalin, thereby reducing urease activity, may reduce NH_3 emission by 50% (Ogink and Kroodsmas, 1996). The efficiency of reducing the release from slats will never exceed 60%, as about 40% of the total NH_3 emission from a building with a slatted floor is from the slurry stored in the channels or pits below the floor.

2. Deep Litter

a. Transfer of Ammonia Cattle urine will infiltrate the deep litter (sawdust or straw), thus, reducing the surface area in contact with the air. Straw also has the effect of reducing the airflow over the emitting surface. Furthermore, deep-litter cattle houses are, in general, naturally ventilated and the transfer of NH_3 from the house to the free atmosphere may differ from mechanically ventilated dairy cow housing often resulting in a cooler environment in the naturally ventilated house (Groot Koerkamp *et al.* (1998b).

Emission may also be limited because a significant fraction of the TAN mineralized from the easily metabolizable N fractions in urine and dung can be absorbed through cation exchange processes by the straw and transformed into organically bound N by microorganisms (Henriksen *et al.*, 2000a). This would suggest that the potential for N losses via volatilization of NH_3 from deep-litter systems might be small due to the immobilization of NH_4^+ . However, O_2 diffuses into the porous surface layer using straw as channels and the O_2 is utilized by aerobic microbial activity in the deep litter, which may cause a temperature increase to about 40–50°C at 10 cm depth. The increase in temperature will induce an upward current of air. As a result, NH_3 losses from deep-litter systems are up to 10% of the N that is excreted and collected in the straw litter (Rom and Henriksen, 2000).

Deep-litter housing systems are mainly used in less intensive production systems with focus on animal welfare, where the animals may be fed less N. This practice will reduce NH_3 emission per livestock unit because TAN excretion is also low per livestock unit.

Generally, a straw-bedded cattle house is likely to emit less NH_3 than a slurry-based, solid-floor cubicle house with automatic scraper. The NH_3 emission is likely to be related to straw (sawdust) usage, downward urine transport, and to the degree of aerobicity (or anaerobicity) in the bedding.

b. Gross Emission Factors Ammonia emissions have been compared between beef cattle on straw-bedded systems and cattle in slurry-based systems (Chambers *et al.*, 2003). This comparative study used replicated forced-ventilated temporary cattle buildings. Therefore the absolute emission factors should be treated with caution. However, the straw-bedded system resulted in significantly less NH₃ emission ($p < 0.10$) than the slurry system, (20.1 kg compared with 29.6 kg NH₃-N per 500 kg liveweight gain, equating to 33 and 49 g NH₃ cow⁻¹ day⁻¹, respectively).

Demmers *et al.* (1998), measured NH₃ emissions equating to an NH₃ emission factor of 19.5 g cow⁻¹ day⁻¹ from beef calves and yearlings in a straw-bedded building. Whereas Oldenburg (1989, cited in Amon *et al.*, 2001) measured lower emission factors from an alpine cattle system (4–10 g LU⁻¹ day⁻¹).

c. Reduction Measures An increase in straw use by 25% from 3.5 kg cow⁻¹ day⁻¹ reduced emissions by 55%. Increasing straw use by 50 or 100% did not result in any additional reductions in emission. Targeted use of additional straw, for example, at the feeding face and around drinking troughs also significantly reduced NH₃ emissions.

The type of bedding material may influence infiltration rate, airflow over the emitting surface, and absorption of liquid effluent (influencing ammonium immobilization). Jeppsson (1999) measured emissions from growing bulls on different bedding types. Ammonia emission factors were 58, 46, and 32 g cow⁻¹ day⁻¹ for the long straw, chopped straw, and peat and chopped straw treatments, respectively.

Within animal welfare constraints, buildings with a greater stocking density would reduce the NH₃ emission per cow. Dietary modification to reduce N excretion would reduce the ammonium pool and thus reduce the potential NH₃ emissions from animal buildings (as well as other stages in the manure management, for example, storage and land spreading).

B. PIG HOUSING

1. Slatted Floor

a. Release and Transfer Ammonia emission from pig housing varies greatly because of differences in surface area of slurry in slurry channels, soiled floor and slat area, slurry pH, slurry TAN concentration, temperature, and ventilation rate (Aarnink *et al.*, 1996; Ni *et al.*, 1999).

It is generally conceded that in buildings with partially slatted floors the majority of the emission is derived from the slurry channels and floor emissions account for between 11 and 40% of the emission from the pens,

the variation being related to variation in the animals soiling the solid floor and size of the slatted area (Aarnink *et al.*, 1996; Hoeksma *et al.*, 1992).

The magnitude of soiled area is related to the animal behavior, which can be controlled partly through design of pens, position of feeders and drinkers, and indoor climate. Therefore, pig behavior has to be accounted for in models depicting release of NH_3 from pig buildings. It has been observed that pigs prefer to defecate/urinate with their back end to a wall, and particularly to the back wall of the pen furthest away from the lying area (Peirson and Brade, 1999; Randall *et al.*, 1983). The pigs seek seclusion for excretory behavior because of their unstable position during this activity (Baxter, 1982).

Normally, in ventilated buildings the pigs prefer to lie on a warm floor that is solid (Peirson and Brade, 1999; Randall *et al.*, 1983), which contribute to a tendency for dunging in the slatted floor area. Thus, fattening pigs (30–110 kg) spent 87% of their time lying, mostly on the solid concrete floor in buildings with a partially slatted floor (Aarnink and Wagernans, 1997). Further, the pigs spent ~44% of their lying time on the solid wall side of the concrete floor, approximately 40% on the partition side of the concrete wall, 13% on the solid wall side of the slatted floor, and 2% on the partition side of the slatted floor (Aarnink and Wagernans, 1997; Aarnink *et al.*, 1997a).

However, at high ambient temperatures, pigs prefer to lie on a cool surface, which will be the slatted floor and in consequence dung on the warmer (previously lying) surface. This fouling causes an increase in the emitting area, not only from the floor but also to some extent from the fouled animals themselves (Aarnink *et al.*, 1995). Pigs spend the least time lying on the slatted floor where the house is cooled with a conventional arrangement of ventilation through a perforated ceiling and where the ventilation system is configured to introduce air through the slatted floor into the room, and during the winter they spend less time on the slatted floor than during the summer (Aarnink and Wagernans, 1997; Aarnink *et al.*, 1997a).

The number of pigs lying on the slatted area and the number of urination and defecation events taking place on the solid concrete floor increase toward the end of the fattening period (Aarnink *et al.*, 1996; Hacker *et al.*, 1994) due to lack of space and increased heat generated by the pigs themselves as they grow bigger. Furthermore, there is a clear diurnal pattern in the activity of pigs; fattening pigs show a small peak in activity and urination in the morning and a larger, broader peak in the afternoon (Aarnink and Wagernans, 1997; Aarnink *et al.*, 1995). Pig activity will increase due to lights being switched on and off and with farm staff entering the building, either to

provide feed or scrape manure alleys (Aarnink *et al.*, 1995; Burton and Beauchamp, 1986).

Model calculations should include seasonal variations, growth, and feed intake of pigs and parameters such as surface area of stored slurry, area of soiled surfaces in the barn, ventilation, TAN, and pH (slurry and soiled floor surfaces). Also, the pH used in the dynamic modeling of NH₃ emission from housing should be chosen with care as surface pH of the slurry differs significantly from the bulk pH (Canh *et al.*, 1998a). Further ventilation may affect NH₃ emission through the transport from the house, also because a sudden increase in ventilation will increase pH due to a release of CO₂ immediately after the change in ventilation rate (Ni *et al.*, 2000).

b. Gross Emission Factors A major factor influencing NH₃ emission from buildings housing fattening pigs is the increase in feed intake during the growth period. Increasing feed intake in the growing period of rearing pigs (10–25 kg) and fatteners (25–110 kg) will increase excretion of TAN and this will lead to a greater emission of NH₃. Mean NH₃ losses per livestock (LU) are larger from pig housing systems than from dairy cattle housing systems, due to a greater amount of TAN in the slurry and a higher temperature in pig houses.

Measured emission of NH₃ from pigs on a fully slatted floor housed in forced-ventilated buildings is conventionally used as the standard emission factors for different pig classes, the emission being given in NH₃ per livestock unit. The loss of NH₃ from pig housing systems with slatted floors range from 17% of total N for piglets to 29% of total N for rearing pigs (Oenema *et al.*, 2001; Poulsen *et al.*, 2001). Instead of relating the emission to the animal or livestock unit, the emission has to be given in relation to TAN in the source (Table V).

c. Reduction Measures Reducing the surface area of the slatted floor may reduce NH₃ emission (Fig. 11), but due to fouling of the solid floor the emission is not always reduced linearly with the reduction in slatted floor area. Pen fouling increases toward the end of a growing period, which will also increase emission due to an increased surface area emitting NH₃ (Aarnink *et al.*, 1995). However, variation in NH₃ emission can be accounted for in terms of the degree of soiling of the solid concrete floor rather than the quantity of slurry stored beneath the slats in partially slatted systems.

It has been shown that distance from slats to the surface of slurry in slurry channel has no or little effect on NH₃ emission rate, if the slurry channel walls are vertical (Ni *et al.*, 1999), because the slurry surface area is similar in a filled and in an empty slurry channel. Therefore, emptying a slurry channel

Table V
Ammonia Emission Factors for Pig Buildings (Aarnink *et al.*, 1996; Groenestein 1994; Groot Koerkamp *et al.*, 1998b; Mannebeck and Oldenburg, 1991; Oenema *et al.*, 2001)

Animal category	Pen design	Emission factor (% of total-N)		Emission factor (kg NH ₃ -N per kg TAN ^a)	
		Slatted floor and slurry	Littered floor	Slatted floor and slurry	Littered floor
Sows	Partially slatted floor and strewed solid floor	12	16	0.16	0.33
Sows	Strewed solid floor		16		0.33
Sows	Fully slatted floor	20		0.26	
Weeners and fatteners	Fully slatted floor	16		0.25	
Weeners and fatteners	Partially slatted floor	8–16 ^b		0.18	

^aTAN = NH₃ + NH₄⁺.

^bRelated to slatted floor area (see Fig. 11).

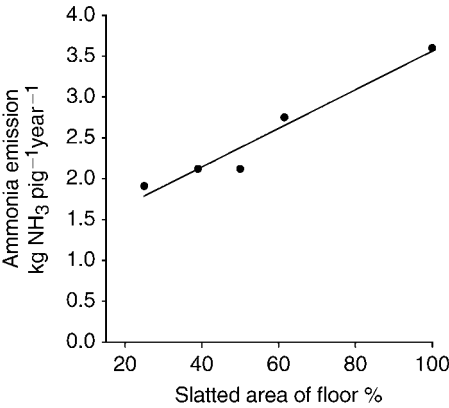


Figure 11 Ammonia emission from pig buildings with partially to full slatted floor (From Aarnink *et al.*, 1997b).

frequently and flushing the channel with water or the liquid fraction of separated slurry may only reduce emission of NH₃ by 20–28% (Aarnink *et al.*, 1995; Hoeksma *et al.*, 1992). In contrast, frequent emptying of slurry channels having inclining walls will reduce NH₃ emission by up to 50% because the surface area of the slurry is reduced due to lowering the height of slurry (Groenestein and Montsma, 1993). However, in a comparison of

three flushing systems, it was found that systems in which a stagnant 10 cm layer of flushing liquid acted as a buffer and a flushing frequency of 1–2 times a day gave lower NH₃ emissions than the system with a sloping channel and a flushing frequency of 6 times a day (Monteny, G. J., personal communication). The largest reduction in emission was achieved where the slurry was discharged from the gutters prior to flushing, resulting in NH₃ emissions about 70% less than those from a fully slatted system.

Cooling of manure stored beneath slatted floors has also been investigated as a method of reducing NH₃ emissions, although results have been inconsistent partly due to low ambient temperatures during the period of the experiment (Andersson, 1998).

2. Deep Litter

a. Transfer of Ammonia Transfers of NH₃ are influenced by the same factors as for cattle in deep-litter systems. As pigs are, in contrast to cattle on deep litter, generally raised in forced-ventilated buildings, ventilation rate and temperature will have a greater influence on NH₃ emission rates. Another factor that appears to influence emission from pig buildings is animal behavior. Pigs have a tendency to defecate and urinate in specific areas, separate from the resting and feeding areas. In deep-litter systems, this can lead to a buildup of dung and urine which can continue to emit NH₃ for a longer period of time than if the dung had dropped through a slatted floor. However, differences in animal behavior and bedding management between studies comparing pigs in slurry and deep-litter systems may be the reason why contradictory results have been observed.

b. Gross Emission Factors Ammonia emission from finishing pigs on deep litter is less than from finishers on slatted floors (Mannebeck and Oldenburg, 1991). However, NH₃ emission from sows on deep litter is greater than from sows on slatted floors. This is challenged by findings showing that from Danish pig fattening housing with deep litter, emissions were 40% (14 g NH₃ pig⁻¹ day⁻¹ or 5.1 kg pig⁻¹ year⁻¹) greater than from fattening pigs on fully slatted floors (Pedersen *et al.*, 1996) but is supported by estimates of emissions from pigs housed on deep litter in Germany which was 75% of the emission from pigs on fully slatted floors (2.3 kg NH₃ pig⁻¹ year⁻¹; Mannebeck and Oldenburg, 1991). From housing of farrowing pigs on deep litter, emission of NH₃ may be as little as 0.8 kg NH₃ pig⁻¹ year⁻¹ (Oldenburg, 1989).

Ammonia emission has been compared between pigs on straw-bedded systems and pigs on slurry-based systems (Chambers *et al.*, 2003). Mean NH₃ losses were significantly greater ($p < 0.05$) from the straw than from the

slurry system, at 7.5 and 5.4 kg $\text{NH}_3\text{-N}$ per 500 kg liveweight gain, respectively. Ammonia emission factors for the straw and slurry systems were 14.7 and 9.4 g $\text{pig}^{-1} \text{ day}^{-1}$, respectively. The greater losses from the straw system were related to the differences in the manure accumulated in specific areas during the housing period. More detailed measurements indicated that emissions were 150 times greater per unit area from the dunging areas than the resting areas used by the pigs (Chambers *et al.*, 2003). The slats allowed the dung and urine to fall into the slurry pit below the house, which was not affected by the airflow within the animal house.

The variation in the reported emissions demonstrate that there is no consistent difference between slurry-based and deep-litter systems. This may be due to differences in addition of straw to the pen, because increasing amounts of straw may reduce the NH_3 volatilization from housed animals (Kirchmann, 1985). In addition, sows are tied and are not able to disturb the deep litter as is the case for finishing pigs on strewn floors, which may cause differences in emission patterns between sows and fatteners housed on deep litter. The discrepancy may also be due to differences in feeding and consequently excretion rate, which has not been reported in most studies.

The nature of the bedding material and the way in which it is treated can also influence NH_3 emission. Groenestein and Van Faassen (1996) compared two sawdust-based materials with emission from a fully slatted floor system. Emissions were reduced in the sawdust treatment where manure was buried weekly without incorporation followed by mixing the top layer (3.5 g $\text{pig}^{-1} \text{ day}^{-1}$), but there was no effect of incorporating weekly into the top 40 cm of the bed (7 g $\text{pig}^{-1} \text{ day}^{-1}$). However, significant N_2O emissions occurred from both treatments. Jeppsson (1998) compared emissions from five different bedding materials for growing-finishing pigs: long straw, chopped straw (with and without a clay mineral additive), wood shavings and a mixture of peat (60%) and chopped straw (40%). Emissions were significantly less with the mixed peat-chopped straw bedding (10.8 g $\text{pig}^{-1} \text{ day}^{-1}$) than the other chopped straw materials (25.1 g $\text{pig}^{-1} \text{ day}^{-1}$). Emissions from the long straw bedding and wood shavings were intermediate (19.3 g $\text{pig}^{-1} \text{ day}^{-1}$).

c. Reduction Measures Emission of NH_3 may be reduced by mixing the top layer once a week with a cultivator. The NH_3 emission is reduced because TAN is depleted due to an increased loss of oxidized N caused by nitrification and denitrification accounting for a loss of 47% of the N excreted (Groenestein and Van Faassen, 1996; Groenestein *et al.*, 1993; Thelosen *et al.*, 1993). This system may be used in some housing systems and then nitrification and denitrification should be included in the calculations. Studies may show that the mixing of straw due to pigs building nests in the deep litter may also enhance nitrification and denitrification.

Increasing the quantity of bedding used in an animal house may result in increased immobilization of NH₄⁺ and a decrease in the airflow over the emitting surface. A doubling of straw use appeared to reduce the NH₃ emission factor per pig by 18% when spread uniformly within the building. Since doubling straw use would increase costs of production, perhaps more targeted use of straw in the building (i.e., in the dunging areas) would result in a similar reduction in NH₃ emissions.

More frequent removal of soiled bedding material would reduce NH₃ emissions from the house, although attention would be needed to reduce emissions during the manure-storage phase.

Increasing the number of animals per pen/room will reduce the relative loss of NH₃ per unit area. However, animal welfare considerations would limit this reduction measure.

VII. AMMONIA EMISSION FROM OUTDOOR AREAS

A. CATTLE FEEDLOTS

1. Transfer of Ammonia

Ammonia emission from the feedlots has been related to several factors including wind speed, surface roughness, and temperature (Bertram *et al.*, 2000). Apart from fences and the animals, few protruding elements affect transfer of NH₃ from the surface to the free atmosphere. In consequence emission may be calculated by using the approach for calculating NH₃ emission from animal slurry applied to fields presented by van der Molen *et al.* (1990a) or Genermont and Cellier (1997). A significant difference, however, is that the infiltration rate of urine into these feedlots will be much less than on cultivated fields, especially if the feedlots are on concrete on which the only infiltration will be via any cracks in the otherwise impermeable surface. Using the information from these studies the NH₃ emission from feedlots should be calculated on an area basis. Input to the model could be urine excreted as it has been shown that feces do not contribute significantly to NH₃ emission (Petersen *et al.*, 1998b). A simple transfer coefficient may be calculated assuming the concentration of TAN in the manure and pH.

2. Gross Emission Factors

A study showed that NH₃ emission per cow was very different between two feedlots; the emission was 0.047 (SD: 0.049) kg NH₃-N cattle⁻¹ day⁻¹ from a 12,000-head of cattle feedlot and 0.1378 (SD: 0.095) kg NH₃-N cattle⁻¹ day⁻¹

from a 25,000-head of cattle feedlot (Bertram *et al.*, 2000). However, expressing emission per unit area showed less difference in emission from the two feedlots (3.53 and 5.35 g $\text{NH}_3\text{-N m}^{-2} \text{ day}^{-1}$, respectively, for the 12,000- and 25,000-head cattle feedlots). The results of this Canadian study were 1.5 and 2.2 kg N $\text{ha}^{-1} \text{ h}^{-1}$, which was very similar to the findings in a US study from 1982, showing an average NH_3 flux of NH_3 from a beef feedlot at 1.4 (SD: 0.7) kg N $\text{ha}^{-1} \text{ h}^{-1}$ as an average of five daytime measurements (Hutchinson *et al.*, 1982). Differences in emission between the three feedlots may be due to differences in animal age and feeding practice.

B. HARDSTANDINGS

1. Transfer of Ammonia

Transfer of NH_3 from the surface of a hardstanding is essentially from a thin emitting layer of excreta. The mass transfer coefficient, K_t , will depend on the surface roughness of the emitting surface and the wind speed. Measurements of emissions from hardstandings on a number of livestock farms (Misselbrook *et al.*, 2001; ongoing measurements unpublished) yielded K_t values in the range 0.0016–0.0260 m s^{-1} (mean: 0.0079, SD: 0.0042 m s^{-1}). No correlation was found between these measurements and ambient wind speed measured at 2 m height close to each measurement site. Actual wind speed at the emitting surface may vary considerably across the yard due to the influence of buildings and other obstructions.

In addition to variation in K_t , NH_3 emission from hardstandings will also depend on the emitting surface area and the TAN content and concentration of the emitting layer. The diet of the animal will influence the subsequent TAN content of the excreta and potentially the pH, thereby influencing the dissociation and release of NH_3 . This will also be influenced by temperatures, which, like wind speed, will vary across the hardstanding due to shading by buildings. The surface area from which emission occurs will be influenced by the behavior of the animals using the hardstanding; urine and feces are unlikely to be deposited evenly across the surface and some areas may receive none. Slope and drainage features of the yard may facilitate removal of some of the urine, but this may also lead to more of the surface area becoming coated with urine. In the same way, scraping will remove an undefined amount of the excreta but will leave a more uniform emitting layer across the whole yard surface. Rainfall may both wash excreta from the yard and possibly facilitate more efficient scraping. For a given unit surface area, therefore, the TAN concentration will depend on the relative dynamics of excreta deposition and removal.

Table VI
Emissions Factors for Hardstandings Used by Livestock

	Emission Factor g NH ₃ -N m ⁻² day ⁻¹	Source
Dairy cattle collecting yard	4.9	Misselbrook <i>et al.</i> , 1998
	6.7	Misselbrook <i>et al.</i> , 2001
Dairy cattle exercise yard	4.3	Keck, 1997
Dairy cattle feeding yard	16.6	Misselbrook <i>et al.</i> , 2001
Beef cattle feeding yard	5.3	Misselbrook <i>et al.</i> , 2001
Sheep handling area	10.6	Misselbrook <i>et al.</i> , 2001
Pig handling area	3.4	Misselbrook <i>et al.</i> , 2001

2. Gross Emission Factors

The spatial variability in the emitting surface and transfer coefficient for hardstandings, as described above, makes it difficult to produce reliable estimates of NH₃ emissions from this source using such algorithms. Emission factors have therefore been derived empirically (Table VI). Few studies have been reported, with the majority of measurements having been conducted in the United Kingdom. These emission factors are expressed on a per unit surface area basis, as measured. Webb and Misselbrook (2004) estimated the amount of TAN deposited on the hardstandings, taking into account the duration of use by livestock and the proportion removed by scraping. For those hardstandings where cleaning was infrequent (less than daily), the emission factor was estimated as 100% of TAN deposited. For dairy cow collecting yards, which were cleaned more frequently, 80% of TAN was estimated to be removed by scraping and the remaining 20% lost via NH₃ emission. Airolidi *et al.* (2000) reported NH₃ emission from a dairy cow exercise yard in Italy of 5% of the total N on the yard surface, approximating to 25% of the urine N.

Misselbrook *et al.* (1998) reported a marked seasonal difference in emission rates from a dairy cow collecting yard from which measurements were made in late summer and winter. This was partly explained by the much greater N content of the cattle urine in summer and also the higher temperatures. Keck (1997) also reported a temperature effect on NH₃ emissions from urine and feces. However, in a larger study covering several farms and different times of year, no seasonal influence was noted on NH₃ emissions (Misselbrook *et al.*, 2001).

3. Reduction Measures

Practical strategies to reduce emissions from hardstandings by increasing the resistance to transport, for example, by covering the emitting surface or

placing barriers around to minimize airflow over the surface, do not exist. Therefore, reduction measures must seek to either reduce the overall emitting surface area or reduce the TAN concentration. Reducing the overall emitting surface area may be achieved by reducing the area allowance per animal. Current studies aim to establish whether the relationship between emission per animal and area allowance is linear. Reducing the TAN concentration may be achieved by effective yard cleaning. Scraping has been shown to be fairly ineffective in this respect, as a thin layer remains on the yard from which emission continues (Braam *et al.*, 1997; Kroodsma *et al.*, 1993; Misselbrook *et al.*, 1998). Washing will both remove TAN from the hardstanding and dilute that which remains and is therefore a more effective reduction strategy (Misselbrook *et al.*, 1998), although the additional slurry volume produced needs to be considered. Regular applications of a urease inhibitor, as has been used on feedlots (Varel *et al.*, 1997), may also reduce emissions by delaying the hydrolysis of urea until after the excreta has entered the store; this is a subject of ongoing studies.

VIII. EMISSION FROM OUTDOOR MANURE STORES

Calculation of NH_3 emission during storage of liquid manure will differ from calculations of emission from solid manure stores. Ammonia emission from liquid manure or slurry should be related to chemistry of the slurry, physics, surface area affected by covers, and climate. The emission from stored solid manure should be related to whether the manure is composting. Prediction of composting may be related to water content, porosity (density) and, C content. Thus, deep litter from pig and cattle housing and pig manure with a large proportion of straw will compost whereas in FYM from cattle the temperature often will not increase (Forshell, 1993). Beef feedlot manure is often so dry that it will not compost without added water and is handled in windrows. In consequence, the calculation of NH_3 emission from stored manure should reflect the variety in manure composition and climate. Further mineralization and immobilization will change the organic N and TAN pool, which will affect emission from the stored manure.

A. SLURRY STORES

1. Transfer of Ammonia

Transport of TAN from the bulk of slurry to the surface of a slurry store is a combination of diffusion and convective movement with liquid that

is moving due to the wind mixing slurry, differences in temperatures in different layers of the stored slurry, and ebullition due to anaerobic degradation of organic components in the slurry. Consequently, the transport of TAN in stored slurry may be 10 times larger than if diffusion was course of the only mode of transport (Olesen and Sommer, 1993). A similar pattern is seen for TAN transport and NH₃ emission from paddy fields fertilized with urea (Leuning *et al.*, 1984).

Surface resistance (r_c , m s⁻¹) has been calculated from studies using wind tunnels for the measurements of NH₃ emission from stored pig slurry and the resistances of the laminar and the turbulent boundary layer were estimated using the algorithms of van der Molen *et al.* (1990a). The estimated coefficients at a wind speed of 2 and 8 m s⁻¹ in the wind tunnel are presented in Table VII showing that a surface crust and a 15 cm straw layer increases the surface resistance significantly. From laboratory studies, Xue *et al.* (1999) proposed that the NH₃ emission may be calculated using the transfer coefficient (K_t) as presented in Table VII. The dynamic chamber studies (Arogo *et al.*, 1999; Xue *et al.*, 1999) give a lower K_t than the K_t estimated using wind tunnel studies at wind speeds of 2 and 8 m s⁻¹ (Olesen and Sommer, 1993), which may be due to a low airflow in the dynamic chamber experiments; a significant resistance due to the laminar layer would not be expected in a dynamic chamber. The transfer coefficients for covered slurry stores calculated using the resistances from the studies where NH₃ emission was measured using wind tunnels and a small dynamic chamber are different as for uncovered slurry (Table VII).

Table VII
Transfer Resistance Coefficients and Transfer Coefficients for Predicting NH₃ Emission Liquid Solution Simulating the Surface Layers of Stored Livestock Slurry (Arogo *et al.*, 1999) from Livestock Slurry Stores (Olesen and Sommer, 1993; Xue *et al.*, 1999)

	r_a s m ⁻¹	r_b	r_c	K_t (m s ⁻¹)	Reference
Uncovered				$0.5\text{--}2.5 \times 10^{-4}$	Arogo <i>et al.</i> , 1999
Uncovered	71–18 ^a	9–22 ^a	18	0.011–0.017	Olesen and Sommer, 1993
Uncovered				0.004×10^{-4}	Xue <i>et al.</i> , 1999
Surface crust	71–18 ^a	9–22 ^a	119	0.005–0.006	Olesen and Sommer
Straw cover	71–18 ^a	9–22 ^a	92	0.006–0.008	Olesen and Sommer
Straw cover				0.0009×10^{-4}	Xue <i>et al.</i> , 1999

^aEstimated at wind speeds of 2 and 8 m s⁻¹.

Table VIII
Ammonia Emission from Uncovered Stored Livestock Slurry (Aneja *et al.*, 2000, 2001; Bode, 1991; Harper and Sharpe, 1998; Harper *et al.*, 2000; Heber *et al.*, 2000; Karlsson, 1996; Sommer, 1997; Sommer *et al.*, 1993; Todd *et al.*, 2001; Zahn *et al.*, 2001)

Animal	Slurry	Store	Emission (kg NH ₃ -N m ⁻² a ⁻¹)	
			Mean	SD
Cattle	Untreated	Concrete store	1.44	0.78
Pig	Untreated	Concrete store	2.18	2.10
Pig	Untreated	Lagoon	0.78	1.07
Cattle and pig	Fermented in biogas plant	Concrete store	2.33	0.68

2. Gross Emission Factors

Ammonia emission from slurry in open tanks, silos, and lagoons ranges from 1.44 to 2.33 kg NH₃-N m⁻² year⁻¹ (Table VIII) corresponding to between 6 and 30% of the total N in stored slurry, assuming there is an emitting surface over the whole year. The NH₃ emission is related to environmental conditions (temperature and wind), slurry composition, and surface area. Losses are larger from pig slurry than from cattle slurry due to differences in TAN content. Emission from pig slurry stored in lagoons is less than that from slurry stored in concrete stores, because the TAN concentration is less in lagoons (Arogo *et al.*, 2003). However, this may be true of emissions per unit area, but because of the greater surface area to volume ratio total losses, expressed as a percentage of TAN, may be as great or greater. Furthermore, emission tends to be twice as large from slurry that has been fermented in a biogas plant than from untreated slurry, because fermented slurry has a higher pH and TAN content (Sommer, 1997; Sommer *et al.*, 1993).

3. Reduction Measures

A cover on the slurry significantly decreases NH₃ loss (Hornig *et al.*, 1999; Misselbrook *et al.*, 2005b; Portejoie *et al.*, 2003; Sommer, 1997; Sommer *et al.*, 1993). The cover may be a natural surface crust formed by solids floating on the surface, a cover of straw, peat or floating expanded clay particles, or a roof. Crust formation will be influenced by both the total content and the nature of the slurry solids; crusting is unlikely to occur on stores with a slurry DM content of <2% and cattle slurries may crust more readily than pig slurries. Covers greatly decrease the air exchange rate

between the surface of the slurry and the atmosphere by creating a stagnant air layer above the slurry through which NH₃ has to be transported by the slow process of diffusion. This decreases the NH₃ losses to less than 10% of those from uncovered slurry. A cover of straw will provide C for the production of VFA, which will contribute to a reduction in pH in the surface of the slurry and thereby reduce NH₃ volatilization (Clemens *et al.*, 2002; Xue *et al.*, 1999).

B. SOLID MANURE STORES

1. Transfer of Ammonia

The transfer of NH₃ away from stored solid manure can be described as for any other NH₃ source by Eq. (1). However, the location of the emitting area varies considerably between different manure types and storage conditions.

In solid manure with low straw content or having a high water content (>50–60%), the diffusion rate of O₂ is low and composting nearly absent (Forshell, 1993; Petersen *et al.*, 1998a) and NH₃ emission occurs exclusively from the outer surface of the stack. Ammonium near the outer surface is depleted by turbulent transport to ambient air, which has a relatively low NH₃ concentration, and is only slowly replenished by mineralization of organic N in this layer. The addition of fresh manure to the surface of the stack prevents further emission from the old outer surface but creates a new outer surface that from which emission can occur. Each fresh addition of manure creates a new pulse of NH₃ emission and in the case of daily additions of manure, a near constant flux of NH₃ into the atmosphere will occur (Muck *et al.*, 1984).

If the manure is porous and there is air access to the base of the stack, self-heating (composting) will occur. In general, composting will start in pig feces, which have a low water content and in heaps of cattle manure with a daily straw addition rate higher than 2.5 kg straw per head of animal. Consequently, composting can lead to the temperature of the stack rising above ambient, and as high as 70–80°C in heaps of manure from buildings with deep litter and manure removed from feedlots at intervals from months to years. This generates a flow of air through the stack, which passes over the large surface area of the stack matrix [A in Eq. (1)]. The concurrent decomposition of organic matter results in a rapid mineralization of organic N to ammonium [NH_{3,G} in Eq. (1)] leading to a rapid and substantial emission. In the absence of forced ventilation, the depth of the composting material is initially 10–30 cm. With time, this increases as the surface dries out and porosity increases. Heaps stacked in one operation will be a source of NH₃

for a few weeks, until the moisture content falls sufficiently to halt decomposition or all the decomposable N has been emitted as NH_3 or oxidized N, or has been converted into organic N. The NH_3 is either transformed to NH_4^+ and adsorbed by the CEC or is lost via volatilization. Active composting is often explicitly a part of manure management, with the aim of reducing the mass and volume of manure to be removed, and to reduce the viability of weed seeds. In such systems, the manure may be turned at periods of 1–3 weeks, to restart composting by bringing moist, undecomposed manure to the surface. Turning of heaps has been shown to increase NH_3 emissions (Parkinson *et al.*, 2004).

3. Gross Emission Factors

During the formation of a manure heap, the temperature inside the heap may increase to 70°C due to aerobic microbial metabolism, that is, composting (Petersen *et al.*, 1998a). Composting generates an upward airflow in the heap and, consequently, fresh air from the atmosphere will enter through the lower section of the heap. Further, composting causes an increase in pH, which increases the NH_3 fraction relative to NH_4^+ . As a result, volatilization of NH_3 from composting solid manure and deep litter may be high (Table IX). Losses of 25–30% of the total-N in stored pig manure and cattle deep litter have been recorded (Karlsson and Jeppsson, 1995; Petersen *et al.*, 1998a), although losses as low as 1–10% have also been measured (Amon *et al.*, 2001; Chadwick, 2005). Rain may leach TAN and thereby reduce NH_3 volatilization (Amon *et al.*, 2001; Chadwick, 2005).

Table IX
Ammonia Emission from Stacked Solid Manure (Amon *et al.*, 2001; Chadwick, 2005;
Karlsson and Jeppsson, 1995; Lammers *et al.*, 1997; Petersen *et al.*, 1998a;
Sommer, 2001; Sommer and Dahl, 1999; Takashi *et al.*, 2001)

Animal	Manure	Temperature >50°C	Emission of NH_3			
			kg $\text{NH}_3\text{-N t}^{-1}$		$\text{NH}_3\text{-N \% of total N}$	
			Mean	SD	Mean	SD
Cattle	FYM	No	0.1	0.1	2.2	1.9
Cattle	FYM	Yes	0.4	0.2	4.9	4.6
Dairy cow	Deep litter	Yes	0.2	0.1	2.3	1.0
	mixed at start					
Dairy cow	Deep litter	Yes	1.3	0.7	15.5	6.5
Pig	FYM	Yes	2.8	0.1	23.5	0.7
Pig	Deep litter	Yes	2.4	0.8	30.2	7.7

4. Reduction Measures

Additions of straw increase the C:N ratio and promote immobilization of TAN (Kirchmann, 1985), but large amounts of straw are required to reduce NH₃ losses. Kirchmann and Witter (1989) calculated that a daily addition of 25 kg straw per cow would be required to reduce NH₃ losses during storage by 50%, and concluded that anaerobic manure storage was superior to aerobic in regard of conservation of manure N during storage. The calculation is confirmed by a laboratory study showing that increasing straw addition from 2.5 to 15 kg straw LU⁻¹ day⁻¹ may reduce emission from 43% of total N to 22% of total-N (Dewes, 1996). Losses can be lowered by 50–90% by decreasing the convection of air through the heap with a cover of tarpaulin or through compaction of the litter (Chadwick, 2005; Sommer, 2001).

IX. PERSPECTIVES

The application of any models developed may be critically constrained by the availability of data needed to run the model. For example, meteorological data, disaggregated to a fine scale, may be readily available to be used in models of emissions that take place in the field. However, data on ambient temperature or windspeed may be of little use to models of emissions from buildings in which temperature, windspeed, and relative humidity will be crucially altered by the shelter provided by the building and also by the metabolic activities of the livestock. In mechanically ventilated buildings ventilation rate often determines NH₃ emissions. While data on ventilation rate may be available for models of emission from individual buildings or farms such data will not be available for national-scale models. Surrogates for ventilation rates may be available based on ambient temperature and windspeed and ambient data may also be used to calculate conditions within naturally ventilated buildings. However, to be accurate such meta-models would require detailed information of the number, age, and weight of animals within buildings and again, this may be available to use for individual buildings or farms but will not be available for national-scale models except via census data of total numbers of livestock, buildings, and averages/distributions of animals within those buildings. Such information is also known as *activity data*, which, in the context of calculating NH₃ emissions, may be defined as data quantifying agricultural practices that have an influence on NH₃ emissions, for example, housing systems.

A sensitivity analysis of the UK National Ammonia Reduction Strategy Evaluation System (NARSES) (Webb and Misselbrook, 2004) model found

that, for this national-scale mass-flow model, 8 of the 10 input data to which the model was most sensitive were these activity data. While most of these activity data related to livestock numbers and their N excretion, both of which may be known with reasonable accuracy at the national level, two other important factors: the length of the housing period for grazing animals and the proportions of livestock housed on slurry- or straw-based systems, were far less certain (Webb and Misselbrook, 2004). It may be concluded that the limiting factor in our ability to model emissions from buildings housing livestock is a knowledge of what is in those buildings and how they are managed.

Process-based modeling is necessary to formulate our understanding of a topic and to identify areas of weakness in our understanding so that future research is properly directed to addressing those weaknesses. In addition process-based models can be an accurate and cost-effective means of estimating emissions from a discrete source. This is especially relevant for predicting or monitoring the impact of emissions from buildings, outdoor yards, and manure stores of a large livestock production unit on adjacent sensitive area(s). The dimensions, characteristics, and animal population of such “fixed” facilities can be accurately determined and hence, if robust and validated models are available, then emission can be reasonably accurately modeled, allowing for seasonal and annual variation in the environmental factors that affect NH_3 emission. However, the adoption of such models for estimating national NH_3 emission involves a number of difficulties, the greatest of which is to obtain sufficiently accurate data on both the physical layout of farm structures and farm management practices (activity data) that influence NH_3 emission. For example, emissions from buildings increase with increasing temperatures (Ni, 1999) and hence emission will be greater in summer than in winter. For livestock, such as pigs and poultry, which are housed all year, this effect can be easily modeled. However, for cattle, which in many countries are housed for 24 h day^{-1} only during winter, there will be confounding between temperature and occupancy. In the early spring and late autumn, cattle may be outside grazing during the day and housed in at night. This practice may extend to early winter and early spring on those farms that practice extended grazing (Webb *et al.*, 2005). In the summer, dairy cattle may enter the buildings for just a few hours per day during the period when they are collected from the fields and brought in for milking. Hence, in order to accurately model the effects of temperature on housing emissions we need not only accurate and disaggregated temperature data (which will be available) but also very accurate data on the length of time that cattle occupy buildings and these data also need to be disaggregated to properly account for any interactions between housing period and climate. At present, such detailed activity data will be available in only a very few countries, if any. We may conclude therefore, that the greatest limitation to

accurately estimating emissions from buildings and stores at the national level is in the paucity or generality of activity data.

The following information is needed to make accurate estimates of national NH₃ emissions from buildings housing livestock, hardstandings, and manure stores.

1. Animal numbers
2. The housing period for all types of cattle and for sheep
3. The amount of time cattle spend on hardstandings and the proportion of cattle that use them
4. The proportions of cattle, all classes, housed on slurry- or straw-based systems
5. The proportions of cattle and pig slurry stored in aboveground tanks, lagoons, and weeping walls
6. The adoption of covers for slurry stores

Many countries have annual surveys of animal numbers and these will be available with an accuracy of <5%, often <2%. The other items will be available from surveys (from Smith *et al.*, 2000, 2001a,b, Webb *et al.*, 2001), however the accuracy of the data will be much less.

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